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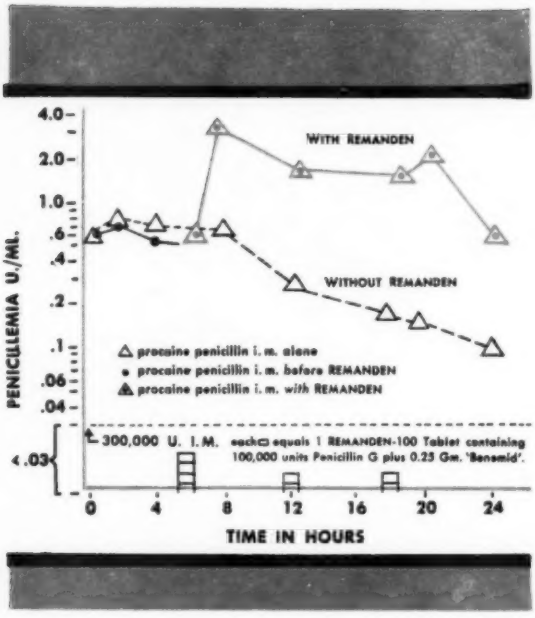

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STUDIES ON RICKETS INDUCED BY A LOW CALCIUM DIET. EFFECT OF STARVATION, CITRATES AND SUCCINATES

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In 1933 Hamilton and Schwartz (1) reported that the addition of acid sodium tartrate to a low phosphorus rachitogenic diet prevented the development of rickets in rats. This observation was extended by Hamilton and Dewar (2) and by Shohl (3), who reported that experimental low phosphorus rickets could be prevented or cured by the dietary addition of citrates as well as tartrates. Furthermore, it was noted that citrates were slightly more effective than tartrates; and the sodium salt of either was more effective than the respective acid in preventing rickets. Shohl (3) found the combination of citric acid with sodium citrate to be more effective than the acid or salt alone in preventing low phosphorus rickets, but could demonstrate no curative action with other organic acids (acetic, lactic, malic, malonic, and succinic) and the sodium salts of these acids. Subsequently, oral citric acid-sodium citrate therapy has been found effective in promoting healing of rachitic infants (4–7).

It has been suggested that the beneficial effect of citrates and tartrates in experimental rickets is due primarily to the formation in the intestinal tract of calcium complexes with these organic acids, thus reducing the formation of insoluble calcium phosphates with a resultant increase in phosphorus available for absorption (2). The formation constant for calcium citrate is slightly greater than that for calcium tartrate, but appreciably greater than those of the other organic acids noted by Shohl (3) to be ineffective in preventing low phosphorus rickets (8). Thus, the concept of chelation in the intestinal tract as the mode of action whereby citrates or tartrates, and not other organic acids, exert a beneficial effect in low phosphorus rickets is consistent with the propensity of the respective acids to form calcium chelates. Others (9), concurring with this concept, have postulated that if the curative action of citrates on experimental or naturally occurring rickets is dependent entirely on the property of this compound to chelate calcium in the intestinal tract, citrates should have no beneficial effect on the rickets induced by *low calcium diets*. The present experiment was designed to determine the presence or absence of any curative action of orally administered citrates on experimental low calcium rickets of rats.

[1] This experiment was performed during the tenure of a postdoctoral fellowship from the Institute of Arthritis and Metabolic Disease of the National Institutes of Health.

PLAN OF THE EXPERIMENT

Production of rickets by low calcium diets. Many investigators (10–15) have induced experimental rickets in rats by feeding low calcium diets. All have indicated that, in the rickets so produced, cartilage cell proliferation and osteoid production are not so extensive as in the rickets induced by feeding low phosphorus diets. No definitive explanation for this difference has been offered, although most authors have noted that weanling rats fed the usual low calcium diets often fail to gain weight, or gain at a much reduced rate as compared to weanling rats fed low phosphorus rachitogenic or stock diets. It has been pointed out that, in the absence of tissue growth, rickets does not develop (16), and this may be a partial explanation of the less severe rickets induced by feeding low calcium diets. The possible effect of alterations in function of the parathyroid glands in modifying experimental low calcium rickets has not previously been emphasized and will be discussed subsequently. In the rickets induced by feeding low calcium diets the serum calcium concentration is low with a normal or slightly elevated serum phosphorus, whereas in experimental low phosphorus rickets serum calcium is normal or slightly elevated, and the serum phosphorus concentration is low (11).

To produce experimental rickets in rats, the ratio of calcium to phosphorus as well as the absolute amounts of these substances in the diet are important in determining the successful production of rickets (15, 17). In the present experiment we wished to provide a low calcium intake and to avoid any deficiency in dietary phosphorus. The diet utilized was the Rachitogenic Diet #2, U.S.P. XII, less the usually added calcium carbonate.[2] To improve growth 5 per cent U.S.P. brewer's yeast powder was added to this diet. The phosphorus in the diet was increased by the addition of 0.55 gm. of mono- and disodium phosphate per 100 gm. of diet. Thus composed, the diet contained 0.04 gm. calcium and 0.84 gm. phosphorus per 100 gm. of feed. The details of the dietary composition, analyses of ash content, and acid and alkali equivalents are recorded in Table I.

Weanling, male, Wistar rats[3] were grouped according to weight in cages having wire mesh bottoms permitting passage of excreta, and fed the low calcium diet for 27 to 35 days. Distilled water was provided ad libitum. The animals were sacrificed by a blow on the head, the proximal tibiae were dissected free and immediately placed in 10 per cent neutral formalin for fixation. If serum was to be obtained, the rats were etherized, bled by aortic puncture (the blood being collected under oil), and proximal tibiae were then obtained after exsanguination. The presence or absence of rickets and any evidence of spontaneous healing was determined by histological study of undecalcified sections of the proximal tibiae.

[2] Obtained from Nutritional Biochemical Corporation, Cleveland, Ohio.
[3] Obtained from Research Supply Co., Philadelphia, Pa.

TABLE I

Low Calcium Rachitogenic Diet

Dietary Components		Contribution Each Dietary Component/100 Gm. Feed			
Constituent	Amount used/100 gm. feed (gm.)	Calcium (gm.)	Phosphorus (gm.)	0.1 N acid (ml.)	0.1 N alkali (ml.)
U.S.P. Rachitogenic Diet #2, less $CaCO_3$	95	.034	.228	226	161
U.S.P. Brewers Yeast	5	.006	.057	31	34
$NaH_2PO_4 \cdot H_2O$	.517	—	.116	67	37
Na_2HPO_4	1.983	—	.434	252	278
Total		0.04	0.835	576	510

Effect of starvation. The curative effect of starvation on experimental *low phosphorus* rickets is well known (16, 18–19). To determine the effect of starvation on experimental *low calcium* rickets and to provide controls for subsequent portions of this experiment, 12 rats, having previously been on the low calcium diet for 25 days, were weighed, divided into groups of three, and subjected to starvation (distilled water ad libitum) for periods of one, two, three, and four days. The rats were then reweighed, etherized, bled by aortic puncture; and after exsanguination the proximal tibiae were dissected free and placed in 10 per cent neutral formalin for fixation.

Effect of citrates and other dietary additions on low calcium rickets. Once it was established that the low calcium diet was rachitogenic, the effect of various salt and acid additions was determined. The additions used were citric or succinic acid, the sodium salts of these acids, sodium bicarbonate, or combinations of these acids with the sodium salts. Succinates had been shown to be ineffective in low phosphorus rickets and were thus selected to compare with the effect of citrates on the low calcium rickets. When mixtures of a salt and acid were used as the dietary addition, the mixtures were so prepared that the proportion of acid to salt was the same as that found most effective by Shohl (3), i.e. 900 ml. of .1 N acid to 600 ml. of .1 N salt. The salts and acids were added to the low calcium diet in the dry state, and the acid or salt equivalents of these additions were calculated on the basis of ml. of 0.1 N acid or salt (3, 20).

Rats were placed on the basic low calcium diet for 26 to 31 days, at which time the dietary additions were incorporated in the feed. The diet and distilled water were given ad libitum, and the weight gain or loss while receiving dietary additions was determined. Initially the salt and acid additions were fed for seven days, at which time the animals were sacrificed. By this time, if healing has occurred, radiological evidence of such can be shown (2); but Park (21) has indicated that changes visible by x-ray lag far behind histological evidence, and beginning healing may be seen at 24 hours in low phosphorus rachitic rats subjected to starvation (22). Harrison and Harrison (23) state that when ef-

fective amounts of citrates are fed to low phosphorus rachitic rats, histological evidence of healing is present at 18 hours after the addition of dietary citrate. Therefore, as any healing due only to the dietary addition should be soon evident, the duration of feeding the various acids and/or salts was reduced to three or four days. The animals were then sacrificed in the same manner as those in the preceding portion of this experiment.

METHODS

Serum calcium determinations were done by the Clark and Collip modification of the Tisdall method (24). Phosphorus analyses were done by the Umbreit modification (25) of the Fiske-Subbarow method (26). The calcium and phosphorus content of brewer's yeast and of the U.S.P. Rachitogenic Diet #2, U.S.P. XII (without the added $CaCO_3$) was determined by ashing and subsequent analyses according to a method previously published (27). The acid and salt equivalents of the basic diet and dietary supplements were determined from the tables of Sherman and Gettler (20). The acid and salt equivalents of brewer's yeast were determined from the following analysis which was compiled by Dr. C. E. Bills (28): Mg. 0.15 gm.%, K. 1.86 gm.%, Na. 0.115 gm.%, Cl. 0.30 gm.%, S. 0.40 gm.%, Fe. 0.02 gm.%. All acid and salt equivalents are reported here in terms of 0.1 N acid or salt, as was done by Shohl (3). The undecalcified histological bone sections were prepared by a modification of the method of Axelrod (29). The degree of rickets was graded as mild, moderate, or severe; and the amount of healing was graded 1+ to 4+, with 1+ representing minimal evidence of healing and 4+ complete healing of the rickets.[4]

RESULTS

Production of low calcium rickets. In Table II are shown the results obtained with 22 rats fed the low calcium diet for periods of 27 to 35 days. The average weight gain on this diet was 1.1 gm. per day, which compares favorably with the weight gain obtained in rats on a high calcium–low phosphorus rachitogenic diet (Rachitogenic Diet #2, U.S.P. XII) (30). In 20 of the 22 rats histological bone sections showed moderate rickets. Two of the rats showed only mild rickets, and in both there was slight evidence of spontaneous healing at the time of sacrifice. The weight gain of these two rats averaged only 0.3 and 0.4 gm. per day, which was appreciably less than that of the other rats of this group; and this failure to grow probably explains the mild degree of rickets. Comparative views of the rickets produced on this low calcium diet and that produced on a high calcium-low phosphorus diet are shown in Figures 1 and 2. To be noted is that the cartilage cell proliferation and osteoid production are not so extensive in the rickets produced by the low calcium diet as by a low phosphorus diet (Rachitogenic Diet #2, U.S.P. XII). Moreover, in the low calcium rachitic section there are many areas of increased bone resorption; and in the metaphyseal area there is extensive proliferation of fibrous tissue which is not present in the low phosphorus rachitic section. These latter two characteristics, which were present in all of the bone sections from the low

[4] The criteria of "healing" utilized in this experiment applies only to calcification of the cartilage matrix in the zone of proliferative cartilage.

TABLE II

Weight Gain, Degree of Rickets, and Serum Analyses of Individual Rats Fed a Low Calcium Diet

Wt. When Low Ca. Diet Begun (gm.)	No. of Days on Diet	Wt. at Time of Sacrifice (gm.)	Degree of Rickets	Spontaneous Healing	Serum	
					Ca (mg.%)	P (mg.%)
50	27	75	Mod.	0		11.6
50	27	94	Mod.	0	5.4	
50	27	90	Mod.	0	4.6	
50	27	5	Mild	1+		5.0
50	35	101	Mod.	0	5.8	10.7
53	35	107	Mod.	0	6.5	12.1
53	35	94	Mod.	0	6.1	15.7
53	35	69	Mild	1+	6.1	5.9
53	35	95	Mod.	0		7.0
48	33	90	Mod.	0		
52	33	85	Mod.	0	6.8*	8.1
54	33	89	Mod.	0		
58	33	134	Mod.	0	7.3	8.2
59	33	107	Mod.	0	5.8	9.6
52	33	82	Mod.	0	6.6	7.7
48	32	78	Mod.	0		
51	32	78	Mod.	0		
52	32	77	Mod.	0		
52	32	83	Mod.	0		
54	32	96	Mod.	0		
50	29	91	Mod.	0		
53	29	94	Mod.	0		

* Indicates pooled sera.

calcium rachitic rats, are strikingly reminiscent of those seen in rats injected with parathyroid extract (31).

The serum calcium of 12 of these rachitic rats averaged 6.1 mg. per 100 ml., and the serum inorganic phosphorus of 13 rats averaged 9.2 mg. per 100 ml. These values are in agreement with those previously published for rats maintained on a low calcium diet (11). Overt tetany was not seen in this group of rats, although Shohl (15) has reported that a latent tetany exists in the low calcium rachitic rat which may be demonstrated by measuring the increased response to electrical stimuli.

Effect of starvation on low calcium rachitic rats. In Table III it is seen that on starvation of rats made rachitic by feeding the low calcium diet for 25 days, histological evidence of healing was not present at 24 hours, but was present after 48 hours of starvation. This healing progressed so that it was virtually complete by the fourth day of starvation. During the period of starvation weight loss was marked and progressive. Analyses of pooled sera from these animals showed that during the course of starvation there was a slight increase in the concentration of serum calcium and an increase followed by a decline in the serum inorganic phosphorus concentration.

TABLE III

Effect of Starvation

No. of Rats	Duration of Starvation (days)	Average Wt. Loss during Starvation (gm.)	Serum Analysis		Degree of Rickets and Healing
			Ca (mg.%)	P (mg.%)	
12	0	Control	6.1†	9.2†	Moderate, no healing
3	1	8	5.8*	11.1*	Moderate, no healing
3	2	14	6.8*	11.2*	Moderate, mod. healing
3	3	22	6.8*	9.3*	Moderate, marked healing
3	4	29	7.7*	9.2*	Mild, healed

* Determinations made on pooled sera from each group.

† Average concentration in 12 rats.

Effect of citrates and other dietary supplements. The results recorded in Table IV indicate that when a mixture of citric *or* succinic acid and the sodium salt of the respective acid was added to the diet in large amounts (equivalent to 1800 ml. .1 N acid and 1200 ml. .1 N salt) and fed for periods of four or seven days, moderate healing of the rickets occurred. If a mixture of succinic acid and sodium bicarbonate was incorporated in the diet and fed for seven days, again healing of the low calcium rickets occurred; but no significant healing occurred if this same mixture (1800 ml. .1 N acid and 1200 ml. .1 N salt) was fed for only four days. Thus, the succinic acid-sodium bicarbonate mixture was less effective in promoting healing than the succinic acid-sodium succinate mixture; and a possible explanation for this difference will be discussed subsequently. In each group of rats in which healing of rickets occurred, there was a marked weight loss (average of 9.0 to 19.5 gm. per rat) during the period of feeding the acid-salt mixture, despite the daily ingestion of 4 to 9 gm. of diet per rat. If the amount of acid and salt addition (whether citrates or succinates) was reduced by one-half (to 900 ml. .1 N acid and 600 ml. .1 N salt) and fed for periods of three to four days, no significant healing was evident at the time of sacrifice. *This latter amount of citric acid-sodium citrate mixture was the same that Shohl (3) had found most effective in preventing or curing experimental "low phosphorus" rickets.*[5] Lesser amounts of the mixtures of citrates or succinates were also ineffective when fed to the low calcium rachitic rats for periods of three to four days.

The results obtained may be summarized as follows: when low calcium rachitic rats were fed mixtures of citric *or* succinic acid with the sodium salt of the respective acid, healing of the rickets occurred only when large amounts of the acid-salt mixture were fed; and the succinate mixture was as effective as the citrate mixture. *There was a marked weight loss in all of the animals in which healing occurred.*

[5] Actually Shohl (3) reported this mixture as effective in *preventing* low calcium rickets in a single group of rats, but the rickets of the control rats was minimal (1+); and the effect of the supplement on the weight gain of the three rats was not reported.

TABLE IV

Effect of Feeding an Organic Acid with a Sodium Salt

Number of Rats	Days on Low Calcium Diet	Diet Supplement	Equivalents of Salt or Acid in Diet Supplement /100 Gm. Diet		Days Diet Supplement Fed	Average Wt. gain (+) or Loss (−) on Diet Supplement (gm.)	Serum		Degree of Rickets	Healing
			ml. .1 N salt	ml. .1 N acid			Ca (mg. %)	P (mg. %)		
14	27	Citric acid Sodium citrate	1200	1800	7	−19	6.1*	7.7*	Moderate	3+ → 4+
3	28	Citric acid Sodium citrate	1200	1800	4	−12			Moderate	2+ → 3+
9	27	Succinic acid Sodium succinate	1200	1800	7	−13	6.9*	6.6*	Moderate	4+
3	28	Succinic acid Sodium succinate	1200	1800	4	−9			Moderate	3+
3	28	Succinic acid Sodium bicarbonate	1200	1800	7	−11			Moderate to severe	3+
3	28	Succinic acid Sodium bicarbonate	1200	1800	4	−3			Moderate	0 → 1+
4	28	Citric acid Sodium citrate	600	900	3	−15			Mild to moderate	0 → 1+
4	28	Succinic acid Sodium succinate	600	900	4	−7			Moderate	0
3	26	Succinic acid Sodium bicarbonate	600	900	3	−5	5.6†	8.1†	Moderate	0
5	31	Citric acid Sodium citrate	300	450	3	+2			Moderate	0
5	31	Succinic acid Sodium succinate	300	450	4	+3			Moderate	0

* Average value from determinations on 9 rats.

† Analysis done on pooled sera.

In Table V are reported the results obtained with the rachitic rats fed additions of *either* the acid *or* sodium salt for three to four days. Succinic acid or citric acid when fed for four days in amounts equivalent to 1800 ml. of .1 N acid caused moderate healing (3+), but feeding of lesser amounts (900 ml. .1 N succinic acid or 1200 ml. .1 N citric acid) did not result in healing of the rickets. The rachitic rats given sodium citrate or sodium succinate in amounts equivalent to 1800 ml. .1 N salt showed only slight (0 to 2+) healing at the time of sacrifice and lesser amounts (1200 ml. .1 N salt or less) were ineffective in

TABLE V

Effect of Feeding an Acid or Salt

Number of Rats	Days on Low Calcium Diet	Composition of Diet Supplement	Equivalents of Salt or Acid in Diet Supplement/100 Gm. Diet		Days Fed Diet Supplement	Average Wt. Gain (+) or Loss (−) on Diet Supplement (gm.)	Degree of Rickets	Healing
			ml. .1 N salt	ml. .1 N acid				
4	28	Succinic acid		1800	4	−4	Moderate	2+ → 3+
3	28	Citric acid		1800	4	−15	Mild to mod.	3+
3	26	Succinic acid		900	3	+1	Moderate	0
3	26	Citric acid		1200	3	−1	Moderate	0
3	28	Sodium bicarbonate	3000		4	−5	Moderate	3+
4	28	Sodium bicarbonate	1800		4	−3	Mild to mod.	3+
4	28	Sodium succinate	1800		4	+4	Moderate	0 → 1+
4	28	Sodium citrate	1800		4	−6	Moderate	1+ → 2+
3	28	Sodium bicarbonate	1200		4	+3	Mild to mod.	0 → 1+
3	28	Sodium succinate	1200		4	+1	Mild to mod.	0
3	28	Sodium citrate	1200		4	−1	Mild to mod.	0
5	31	Sodium bicarbonate	600		3	+1	Moderate	0
5	31	Sodium bicarbonate	300		3	+2	Mild to mod.	0

producing healing. On the other hand, rats fed sodium bicarbonate in an amount equivalent to 1800 ml. .1 N salt showed much more extensive healing than did the rats receiving equivalent amounts of sodium succinate or citrate. In the rats receiving either the salt or acid addition diet intake was satisfactory, and the weight loss was not so marked as in the previous rats receiving combinations of salt and acid.

Thus, in summary: rachitic rats fed large amounts of succinic or citric acid (equivalent to 1800 ml. .1 N acid) showed evidence of moderate healing of the rickets at the time of sacrifice; but equivalent amounts of the sodium salts of these acids produced only minimal evidence of healing, the succinates (either the acid or salt) being as effective as the citrates. Some loss of weight occurred in all of those rats showing significant healing at the time of sacrifice.

DISCUSSION

Experimental low calcium rickets. Moderate rickets of rather uniform degree was produced by use of a low calcium-high phosphorus diet. Rats fed a high calcium-low phosphorus rachitogenic diet having a similar weight gain usually exhibit a more severe degree of rickets than was seen in the rats fed the low calcium diet in these experiments. Although, as mentioned previously, reduced rate of growth may have been a factor in the failure of previous investigators to produce uniformly severe rickets by feeding low calcium diets

(10–15), additional factors warrant consideration. Metabolic acidosis tends to intensify and metabolic akalosis to lessen the development of experimental rickets (32–33). On this basis, the low calcium-high phosphorus diet (an acid ash diet) should have proven superior to a high calcium-low phosphorus diet (an alkaline ash diet) in the production of experimental rickets. As the converse was true, the acid or alkaline ash of the two types of diets affords no explanation for the less severe rickets induced by low calcium diets.

It is known that enlargement of the parathyroid glands occurs in animals fed calcium deficient diets (34–35). Circumstantial evidence suggests that this enlargement signifies either an actual increase in output or an effort on the part of the glands to increase the output of parathyroid hormone (36–37). The striking resemblance of the metaphyseal area in the bone sections from the low calcium rachitic rats to that seen in experimental hyperparathyroidism of rats (31) suggests an increased parathyroid hormone effect in these rachitic animals. This apparent secondary hyperparathyroidism would seem an additional factor modifying the histology and severity of experimental low calcium rickets.[6]

Effect of starvation. Just as in experimental low phosphorus rickets (18), starvation of low calcium rachitic rats resulted in healing. Whereas a healing effect on low phosphorus rickets is evident histologically after 24 hours of starvation (22), a healing effect on low calcium rickets was not evident until after 48 hours of starvation. The changes observed in serum calcium and phosphorus concentrations during starvation of the low calcium rachitic rats differ from those occuring on starvation of low phosphorus rachitic rats (16, 19). In both types of rickets starvation resulted in an abrupt increase in serum phosphorus concentration, and this increase probably represented phosphorus derived from protein catabolism. The slight changes in the low serum calcium concentration on starvation of low calcium rachitic rats contrasts with the marked decrease in serum calcium of similarly starved low phosphorus rachitic rats. In both types of rickets healing was in progress during the period of starvation. This may explain the persistence of low calcium concentrations, for non-rachitic rats subjected to starvation maintain an essentially normal serum calcium concentration (16). The increase in serum phosphorus concentration occurring on starvation of low phosphorus rachitic rats has been offered as an explanation for the healing effect of starvation on such animals. The different calcium and phosphorus concentrations in low calcium rickets, and the relatively slight changes in these concentrations induced by starvation, may account for the slower healing rate noted in the low calcium rachitic rats.

[6] To date we have been unsuccessful in producing experimental low calcium rickets in parathyroidectomized rats. Most of the parathyroidectomized rats fed the low calcium diet have died in tetany, and the few survivors have failed to grow.

Since starvation with its attendant weight loss has a curative action on the rachitic process, the presence or absence of weight loss must be considered in evaluating the effectiveness of various regimens in producing healing of experimental rickets.

Effect of citrates, succinates, and bicarbonates on low calcium rickets. Although the dietary addition of large amounts (comprising 25 per cent of the diet) of a citric acid-sodium citrate mixture or a succinic acid-sodium succinate mixture induced healing of the low calcium rickets, there was a marked weight loss in all rats receiving such mixtures. The succinate mixture proved as effective as the citrate mixture, and the weight loss with either acid-salt addition was similar. Because of this marked weight loss, no curative action may be ascribed to either the succinate or citrate mixture per se, for starvation with a *comparable weight loss* also induces a healing effect. The large amount of sodium present in the effective mixtures may have induced sufficient water retention to mask even greater weight losses than those recorded.

The dietary addition of large amounts of citric or succinic acid alone induced healing of the low calcium rickets; but here, too, weight loss occurred. As only a slight weight loss occurred with this succinic acid addition (1800 ml. .1 N acid), some factor other than weight loss probably contributed to the healing effect. Previous observations on the beneficial effect of increased intestinal acidity on experimental rickets (2–3, 32–33) could be invoked as an explanation for these results. However, the large amounts of organic acids administered may also have induced some degree of metabolic acidosis, a state reported to increase the severity of experimental rickets (2–3, 32–33).

Feeding of sodium citrate or succinate in amounts equivalent to effective amounts of the acid resulted in only slight healing of the rickets. The administration of equivalent amounts of sodium bicarbonate, a more alkaline salt, caused extensive healing. In these animals receiving sodium salts water retention may have occurred, thus masking actual weight loss. Moreover, as with the acid additions, opposing factors may have been operative. Alkalinity of the intestinal contents has been reported to intensify experimental rickets, whereas metabolic alkalosis exerts a curative action (2–3, 32–33). Thus, in the present experiment, a combination of factors may account for the slight curative effect induced by the salt or acid additions.

In this experiment we were unable to demonstrate any specific curative action of citrates in experimental low calcium rickets of rats. Balance studies on healthy adults receiving complete diets have shown that oral administration of citrates is ineffective in increasing calcium absorption (38), and citrates orally do not increase serum citrate concentrations in rachitic children (7). In *normal* rats, furthermore, citrate feeding does not increase the calcium content of the carcass (39). Recently, Steenbock and coworkers (40–41) have shown that the dietary addition of citrates to rats receiving a high calcium-low phosphorus rachitogenic diet *reduces* calcium absorption. Furthermore, they

suggested that the chelation of calcium by citrate in the intestinal tract permits an increase in phytate hydrolysis, thus rendering additional phosphorus available for absorption. In a single group of rats made rachitic by feeding a low calcium-high phosphorus diet these authors (40–41), also, were unable to demonstrate any curative effect from orally administered citrates.

In experimental low phosphorus rickets, where the availability of phosphorus for absorption rather than calcium is the critical factor, the curative action of citrates is well established (2–3), 9–42). Although appreciable quantities of citrate are present in bone (43–45), most of this may be bound at the surface of bone crystals (46). The recognized role of the citric acid cycle in carbohydrate metabolism offers an additional explanation for the presence of citrate in bone, as glycolysis has been deemed necessary for the mineralization of cartilage matrix (47–48). Also, recent evidence suggests that citrates may have an even more specific action in bone metabolism (49–50). Despite these latter observations, however, the data obtained in the present experiment support the concept that any curative action of orally administered citrates in experimental or naturally occurring rickets may be explained by the action of citrates in the intestinal tract; this action, as suggested by others (2, 9, 38), being dependent on the property of citrates to chelate calcium, thereby rendering an increase in phosphorus available for absorption. Thus, where there is little calcium for chelation, as in rickets induced by a low calcium diet, citrate administration is without benefit.

CONCLUSIONS

1. Experimental rickets in rats was induced by feeding a low calcium-high phosphorus diet. The rickets so induced differs from experimental low phosphorus rickets in that in the former there is evidence of more bone resorption, and extensive fibrous tissue proliferation is present in the metaphysis.

2. Starvation of rats made rachitic by feeding the low calcium diet resulted in healing of the rachitic process. As starvation with attendant weight loss has a curative action on the rachitic process, the factor of weight loss must be considered in evaluating the effectiveness of various regimens in producing healing of experimental rickets.

3. The oral administration of a mixture of citric acid-sodium citrate or succinic acid-sodium succinate did not promote healing of the low calcium rickets until such amounts were given (approximately 25 per cent of the diet) as to induce a marked weight loss in the rachitic rats. The succinate mixture was as effective as the citrate mixture, but because of the weight loss with both, *no curative action could be ascribed to either mixture.*

4. Evidence was obtained that the administration of organic acids or sodium salts, irrespective of their propensity to chelate calcium, has a slight healing effect in experimental low calcium rickets.

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EXPLANATION OF ILLUSTRATIONS

Fig. 1. Undecalcified sections of proximal tibiae stained with silver nitrate, hematoxylin, and eosin. (×35). A. Low phosphorus rickets. Note the wide zone of proliferating cartilage cells. Abundant (unstained) osteoid tissue surrounds sparsely calcified (black) trabeculae. B. Low calcium rickets. Cartilage cell proliferation and production of osteoid tissue is reduced as compared to A. In addition, fibrous tissue proliferation is evident in the metaphysial area.

Fig. 2. Same as Fig. 1. A. Low phosphorus rickets. Upper margin continuous with zone of proliferative cartilage. Wide seams of osteoid tissue, irregular calcification, and marrow elements present. Note absence of fibrous tissue (×125). B. Low calcium rickets. Extensive fibrosis throughout metaphysis (×125). C. Low calcium rickets. (×250). Single trabecula showing simultaneous osteoid formation (superior border) and bone destruction (inferior border).

FIG. 1A.

FIG. 1B.

FIG. 2A.

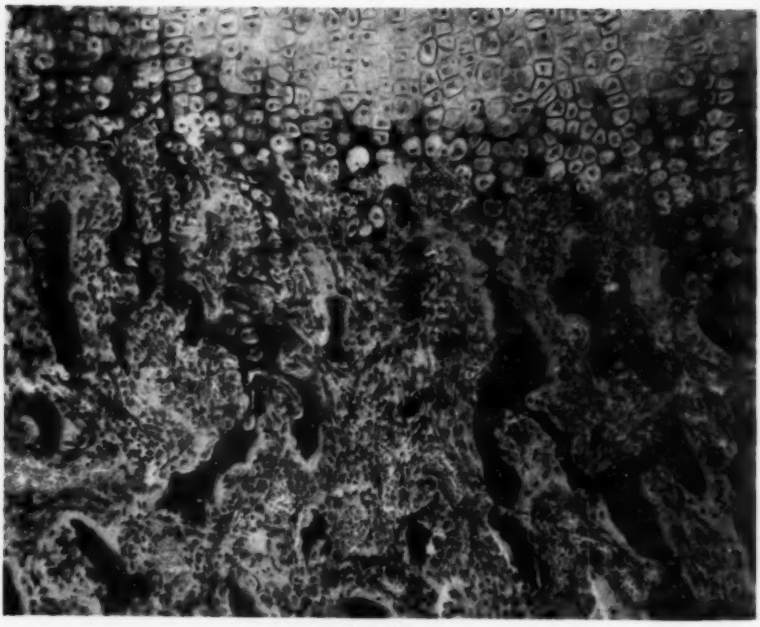

FIG. 2B.

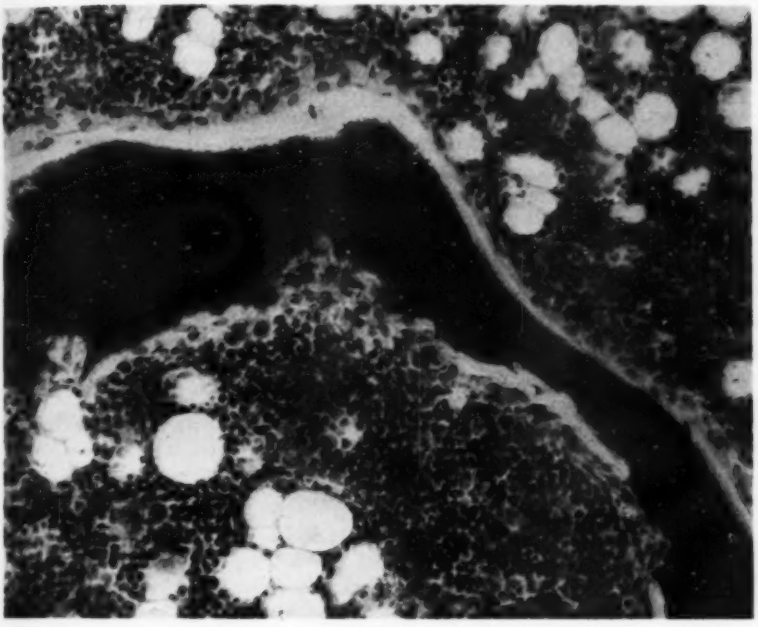

FIG. 2C.

ACQUIRED ARTERIOVENOUS FISTULA COMPLICATED BY PSEUDOMONAS AERUGINOSA ENDARTERITIS AND ENDOCARDITIS

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During a review of the clinical records of patients with *Pseudomonas* bacteremias, attention was focussed on a case in which this organism produced endarteritis in a traumatic arteriovenous fistula, and endocarditis of the aortic valve. Endarteritis is a relatively unusual complication of A-V fistula, only 14 cases having been reported (1–14). In six of these there was associated infection of the endocardium. In none of the previous cases was *Pseudomonas aeruginosa* the causative organism.

CASE REPORT

A 26 year old white housewife (J. H. H. #441128) was admitted to the Johns Hopkins Hospital on October 31, 1947 for chills and fever of 3 months duration.

Four months previously on June 9, 1947, she had undergone surgery at another hospital for ruptured ectopic pregnancy. Because of severe bleeding encountered at operation, hysterectomy was performed. Postoperatively, she was given transfusions of whole blood and her course was uneventful although she remained weak. On July 7, one month after operation, she had the first of several episodes of rigors, hematuria and fever. These recurred every two or three days and on the morning of September 1, she was hospitalized with severe suprapubic pain, urgency and inability to urinate. At cystoscopy on that day, profuse bleeding from the left ureteral orifice was found but retrograde pyelography showed no abnormality of the upper urinary tracts. Laparotomy was performed on September 8. Extensive bleeding was again encountered and detailed exploration was not carried out. There was no cardiac murmur or other localizing sign. Hematologic studies, including sternal marrow examination and tests for coagulation defects were normal.

She continued to have high fever, which failed to respond to the administration of penicillin and streptomycin, although she showed transient defervescence when sulfadiazine was added to the regimen. She developed right pleuritic pain, dyspnea, and hemoptysis and a chest roentgenogram was said to have shown "pneumonia".

During the next few weeks, she developed cardiomegaly, an apical systolic murmur appeared, and increasing hepatosplenomegaly was noted. On October 24, *Ps. aeruginosa* was isolated from the blood for the first time and three additional blood cultures during the next few days were also positive for this organism, which was resistant to streptomycin *in vitro*. She was transferred to this hospital for further studies on October 31.

At this time, she complained of recurrent fever and chills, left upper quadrant pain, fatiguability and anorexia. She had lost 25 pounds.

There was no history of rheumatic disease and no cardiac murmur had been heard prior to the present illness.

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[2] Fellow of the National Foundation for Infantile Paralysis.

Physical Examination. Temperature 104.6°F., pulse 120/min., blood pressure 130/70, respirations 30/min. The patient was acutely ill. The skin and mucous membranes were pale and no petechiae were seen. The ocular fundi were normal. There was splinting of the right chest and dullness and scattered rales were present at the base of the right lung. The heart was slightly enlarged to the left. The cardiac rhythm was regular and a systolic murmur was heard at the left sternal border in the 2nd and 3rd interspaces. The liver was palpable just below the costal margin, and the spleen was easily felt. The fingers were not clubbed. Neurologic examination was normal.

Laboratory Data. The hemoglobin was 12 Gm. per cent; hematocrit 38 per cent, and erythrocyte sedimentation rate 36 mm./hr. (Wintrobe). The blood leukocyte count was 10,240/mm.^{3} with 1 per cent myelocytes, 7 per cent juvenile forms, 78 per cent polymorphonuclear leukocytes and 14 per cent lymphocytes. The leukocytes remained in this range throughout the illness. The urine was acid; Sp. Gr. 1.022; no glucose or protein; sediment contained 5 RBC. and 3–5 WBC. per high power field. During the second week in the hospital she developed proteinuria and the hematuria and pyuria increased. Blood NPN was 22 mgm. per cent on admission and eventually rose to 58 mgm. per cent. Blood glucose, serum calcium, phosphorus, total protein, and albumin-globulin ratio were normal.

Three blood cultures drawn on the day of admission grew *Ps. aeruginosa*. The organism was resistant to greater than 250 units of streptomycin, and was agglutinated by the patient's serum in 1:2560 dilution. Urine obtained by ureteral catheterization was bacteriologically sterile.

Course in the Hospital. The patient had hectic fever up to 105°F. Three days after admission, a continuous bruit, accentuated in systole, was heard over the lower abdomen and over the left femoral artery. This murmur was also audible over the left buttock. Blood pressures in the thighs were equal.

Retrograde pyelograms demonstrated downward displacement of the left kidney, presumably by the enlarged spleen, and moderate hydroureter. In view of the abdominal murmur, the diagnosis of infected aneurysm of the left iliac artery was made and the patient was again subjected to surgical exploration. At operation on November 16, there was a palpable thrill over the left hypogastric artery but extensive dissection did not disclose any aneurysm or pelvic tumor. Compression of the iliac artery produced no slowing of the pulse.

Postoperatively, she was begun on a regimen of penicillin, streptomycin, and sulfadiazine; the last two were soon discontinued because of oliguria. The patient's condition deteriorated rapidly during the next week. Petechiae appeared over the face and trunk, and a blowing diastolic murmur appeared along the left sternal border. She became comatose and died November 25, approximately 5 months after the onset of illness.

Post Mortem Examination. Autopsy (J. H. H. #20932) was performed immediately after death. There were bilateral *pleural adhesions* with multiple *pulmonary infarcts*. The *heart* weighed 290 grams; the myocardium contained numerous small yellow-centered hemorrhages. The tricuspid, pulmonary and mitral valves were normal, but there were huge reddish-brown vegetations adherent to the three aortic cusps (Fig. 1). These vegetations extended one cm. up the aorta and down onto the mural endocardium of the left ventricle. The coronary vessels were patent although the right ostium had been slightly encroached upon by a vegetation.

The *spleen* weighed 800 grams and contained numerous infected infarcts. The *kidneys* were swollen, and contained numerous cortical abscesses. A branch of the mesenteric artery contained a mycotic aneurysm.

There was a 5 cm. mass in the left side of the pelvis adherent to the ureter and composed of a highly vascular, densely scarred adipose tissue. A branch of the hypogastric

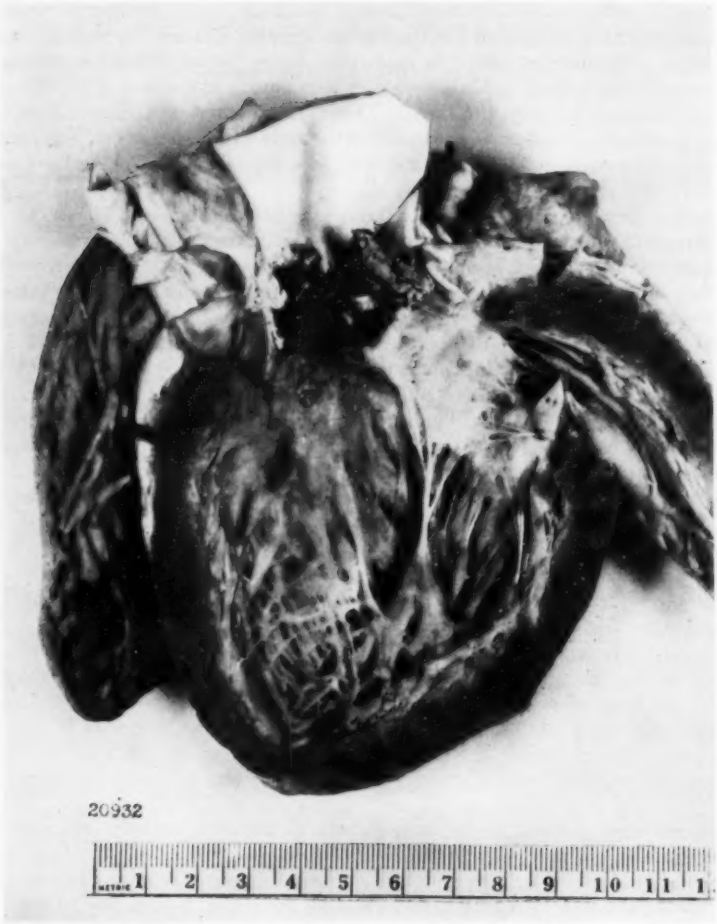

FIG. 1. Gross appearance of the heart revealing large fungating vegetations on the aortic valve. (Photograph by Miss Patricia Whitney.)

artery was found to communicate with a large vein near the left sacroiliac synchondrosis and an aneurysmal sac 2.5 cm. in diameter was adherent to the left ureter as it entered the bladder. The sac contained friable vegetations.

Microscopic examination revealed multiple small myocardial abscesses containing many Gram negative rods. The aortic vegetations were swarming with similar bacilli (Fig. 2).

The lungs, spleen, and kidneys contained numerous infected infarcts in which bacilli could be seen. Histologic sections through the fistula revealed extensive fibrosis of the arterial wall and masses of thrombus containing Gram negative bacilli projecting into the lumen.

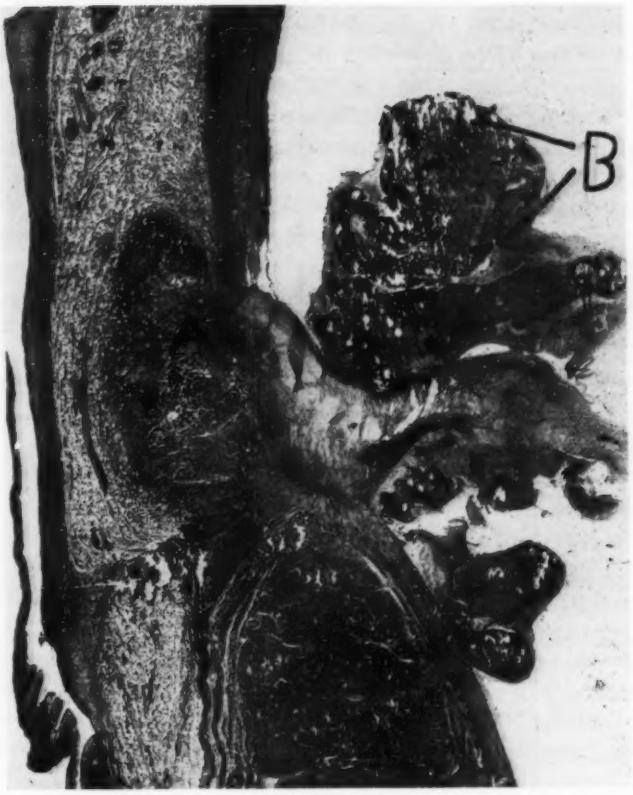

FIG. 2. Section through the aortic valve (Hematoxylin and Eosin stain × 10). A. indicates a valve ring abscess. B. represent clumps of bacteria in the vegetations. (Photograph by Mr. Chester F. Reather.)

Cultures of heart blood, spleen, and the vegetations from the aortic valve and arteriovenous fistula all grew *Ps. aeruginosa* in pure culture.

The final anatomical diagnosis was: *History of extrauterine mass in left pelvis and operative removal of uterus. Bacterial endocarditis of aortic valve (B. pyocyaneus) with mural endocarditis of left ventricle. Infected (B. pyocyaneus) arterio-venous fistula involving a branch of the hypogastric artery. Mycotic aneurysm of mesenteric artery. Widespread abscess formation (B. pyocyaneus). Septic infarcts of lungs and spleen. Superficial ulceration of vagina and esophagus. Tubular degeneration of adrenals. Subacute splenic tumor. Chronic passive congestion, liver. Hydroureter, left. Hyaline nodules in liver. Hyperplasia of vertebral marrow.*

COMMENT

It is unusual for traumatic arteriovenous communications to become infected. For example, of 73 traumatic A-V fistulae studied by Seeley (15), none

TABLE I

Reported Cases of Bacterial Endarteritis Involving Traumatic Arteriovenous Fistulae

Author, Reference, Year	Duration and Site of Fistula	Bacteriology	Heart Valve Involvement	Comment
1. Hamman and Rienhoff, 1935	R. Ext. Iliac 17 years	*St. viridans*	None	Recovery with excision.
2. Porter and Williams 1939	R. Femoral 18 months	*St. viridans*	Aortic	Post mortem.
3. Leaman 1939	L. Thigh 9 years	*St. viridans*	Probable Aortic	Died; no autopsy. Fistula excised with temporary improvement. Recurrence of positive blood cultures.
4. Touroff, Lande, Kroop 1942	L. Femoral 9 years	*St. viridans*	None	Recovered; Rx: Surgery and sulfonamides.
5. Lipton and Miller 1944	L. Thigh 15 years	*St. viridans*	None	Recovered; Rx: Surgery.
6. Cutler and Wolf 1946	R. Femoral 30 years	*St. viridans*	Aortic	Post mortem; Rx: Penicillin and sulfonamides.
7. Shumaker and Welford 1946	R. Thigh 2 months	*St. viridans*	None	Recovered; Rx: Penicillin and surgery.
8. Statland and Orr 1949	R. Thigh 4 years	*St. viridans*	None	Recovered; Rx: Penicillin and surgery.
9. Cohn and Lipsitch	R. Thigh 53 years	*St. viridans*	None	Recovered; Rx: Penicillin and surgery.
10. Williams 1952	L. Axillary 15 years	Hemolytic streptococcus	None	Recovered; Rx: Penicillin and surgery.
11. Heckler and Tikellis 1952	L. Femoral 17 years	*St. viridans*	Aortic	Post mortem. Rx: Surgery. Septicemia cured. Death due to pulmonary and systemic emboli and heart failure.
12. Kahn, Livingston, and Charet 1953	L. Femoral 10 years	*St. viridans*	None	Recovered; Rx: Penicillin, streptomycin, aureomycin, surgery. 6 months later, died. P.M.: Systemic lupus erythematosus.
13. Parmley, Orbison, Hughes, Mattingly 1955	R. Femoral 6 months	*St. fecalis*	Aortic Mitral	Died; Rx: Penicillin, streptomycin, erythromycin. A-V fistula excised. Post mortem.
14. Hook, Wainer, McGee, Sellers 1957	L. Femoral 2 years	*Staph. albus*	Probable Aortic	Died; Rx: Penicillin, surgery. Septicemia cleared. Aortic insufficiency, peripheral emboli, heart failure.
15. Curtin, Petersdorf, Bennett 1957	L. Hypogastric 1 month	*Ps. aeruginosa*	Aortic	Died. Post mortem.

was infected. There have been 14 patients with endarteritis of such a communication described in the American literature. The salient features of these cases are summarized in Table I. *Streptococcus viridans* was the infecting organism in 12, *Staphylococcus albus* in one, and *Str. fecalis* in one. Six of the 14 patients also had endocarditis. There was usually no underlying organic heart disease, and the valvular lesions were invariably aortic. Systemic embolization was common. In contrast, systemic emboli were very rare in the cases of endarteritis without valvular vegetations, although pulmonary embolization was frequent in these patients. Splenomegaly accompanied endarteritis with or without endocarditis. Fistulae of long standing often contained calcified thrombi and several patients were aware of a bruit at the site of the communication. Blood cultures in cases of endarteritis were consistently positive except in a patient

reported by Kahn (12) in whom only aspiration of the femoral vein proximal to the shunt yielded the causative organism.

Lillehei produced acute bacterial infection of the mitral and aortic valves by creating large aorto-caval or bilateral iliac arteriovenous fistulae (16, 17). Seventy per cent of these animals spontaneously developed bacterial endocarditis over a period of weeks or months. Following intravenous administration of bacterial cultures, the lesions developed within a few days. Endarteritis was not a prerequisite for endocarditis; in most animals the shunts were free of infection. Animals with smaller A-V shunts failed to develop valvulitis even after administration of large numbers of bacteria. These findings led Lillehei to postulate that "stress" on the heart produced by the large shunts predisposed the organ to infection.

Experiments by Rabens *et al.* (18) also suggest a definite relationship between arteriovenous fistulae and susceptibility to acute endocarditis. However, the majority of Rabens' animals developed endocarditis only in the presence of arteritis in the communication.

It is well-known that cardiac work is increased in the presence of arteriovenous communications but cardiomegaly and congestive heart failure are late sequelae (19). In some patients in whom A-V fistulae were associated with endocarditis involvement of the valves clearly preceded the appearance of decompensation. Furthermore, bacterial valvulitis occurring as a consequence of other forms of heart disease, is rare in patients with long-standing heart failure (20). Therefore, if "stress" on the heart is a factor in the pathogenesis of endocarditis associated with A-V fistulae, it is clearly of a different nature from the hemodynamic alterations which usually accompany myocardial failure.

The studies of Gowdy and his associates (21) shed some light on the pathogenesis of the acute valvulitis in experimental animals. These investigators demonstrated that lesions varying from minute endocardial hemorrhages to 3–4 millimeter nodules along the borders of the mitral and tricuspid valves appeared within a few days after production of aorto-caval shunts in dogs. Bacterial clearance studies in these animals indicated that organisms are removed from the circulation more slowly than in normal dogs presumably because a large volume of blood was shunted past the portal venous system by the large A-V communication. This factor, in addition to the endocardial lesions, may be of great importance in increasing the susceptibility of the heart to infection. It would be most interesting to assess the effect of A-V communications that do not involve the inferior vena caval system upon endocardial infection.

The most cogent argument that stress *per se* is not the only mechanism influencing the development of endocarditis in man is the fact that valvular involvement in human cases has invariably been associated with infection in

the fistula. This suggests that infection in the heart valves is secondary to a primary focus at the site of the shunt. The improvement which followed fistulectomy in two patients supports this. In contrast, all experimental animals succumbed to endocarditis despite disruption of the arteriovenous communications and antibiotic therapy.

An unusual feature in this case was the isolation of *Pseudomonas* from the blood stream during life and from the heart valves and fistula at necropsy. This organism usually attacks chronically ill or debilitated patients. Transient bacteremia is a frequent consequence of instrumentation of the lower urinary tract. It is probable that the fistula developed after the operation for ectopic pregnancy and became infected as a result of bacteremia during cystoscopy and urethral instrumentation. While the possibility that endocarditis was the primary infection and that the fistula resulted from rupture of a mycotic aneurysm of the hypogastric artery cannot be ruled out completely, it is doubtful. The history of pelvic operation with severe bleeding suggests that the fistula was traumatic. Fever and suprapubic pain with left ureteral bleeding *preceded* the onset of anemia, cardiac murmurs and evidence of infection in the lungs. The pelvic bruit was heard several days before the aortic diastolic murmur appeared. This evolution of the clinical manifestations, together with the rarity of mycotic aneurysms in vessels as large as the hypogastric artery, make the previously suggested sequence far more likely. Although McNeely (22) and later Merritt (23) have reported the occurrence of endocarditis consequent to instrumentation of the lower urinary tract, pre-existing valvular heart disease was present in all but one of their cases.

Pseudomonas is a relatively unusual cause of endocarditis; we have been able to find only 15 previous reports (24–38). The first was described by Barker from this institution in 1897 (24). A summary of the available literature in 1951 revealed 11 patients with acute valvulitis produced by this organism (34). Kenoyer has reported recovery in a patient treated with neomycin (35). Brundson *et al.* described a *Pseudomonas* infection of the mitral valve after commissurotomy (37). *Pseudomonas* is becoming an increasingly troublesome member of the resistant nosocomial flora; it seems probable that secondary infection by this organism will occur more frequently in the future.

SUMMARY

A case of bacterial endarteritis and endocarditis of the aortic valve, complicating arteriovenous fistula, in which *Pseudomonas aeruginosa* was the etiologic agent is described. Chemotherapy failed to control the infection and attempted surgical excision of the A-V communication was unsuccessful. The relationship between experimentally induced endocarditis in animals with arteriovenous shunts and this disease in man is discussed.

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THE RELATIONSHIPS BETWEEN THE CHEMICAL NATURE OF THE ANTIGEN, ANTIGEN DOSAGE, RATE OF ANTIBODY SYNTHESIS AND THE OCCURRENCE OF ARTERITIS AND GLOMERULONEPHRITIS IN EXPERIMENTAL HYPERSENSITIVITY[1]

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The present study was undertaken to determine (a) the quantitative relationship between antigen and antibody necessary to evoke lesions of hypersensitivity and (b) the effect of the chemical nature of the antigen on this relationship and on the character and distribution of the allergic inflammatory lesions. Accordingly, large numbers of rabbits were treated with graded doses of crystallized bovine albumin or bovine gamma globulin, given as a single intravenous injection. This report covers observations made on the rates of antigen elimination and on the development of allergic lesions following the injection of these antigens.

A correlation between antigen dosage and the appearance of allergic symptoms in patients receiving foreign immune sera was noted by several early investigators. Gerlough (1) found that the incidence of serum sickness in humans was directly related to the quantity of serum administered. Rich and Gregory (2) demonstrated that an amount of horse serum many times larger than that ordinarily used for immunization was required for the production of vascular lesions in rabbits. More recently, crystallized bovine albumin at dosages of 0.25 and 1 gm. per kg. of body weight and bovine gamma globulin at a dose of 1 gm. per kg. have been shown to be effective in producing lesions of the serum sickness type (3–5). The only pointed study undertaken to determine the effect of antigen dosage on the development of lesions is that of Moll and Hawn (6) who gave graded doses of bovine gamma globulin to a series of rabbits. When amounts of bovine gamma globulin ranging from 0.25 to 1 gm. per kg. body weight were used, glomerular lesions attributable to hypersensitivity were seen in most animals. These workers further noted that a depression of complement (presumably as a result of its fixation in the interaction of antigen and antibody) regularly occurred in animals developing lesions.

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In the present study, a correlation between the immune response and the development of allergic lesions has been obtained at 4 different dosages of bovine albumin and 3 different dosages of bovine gamma globulin. From antigen elimination curves obtained by quantitative immunochemical analysis, the amount of antigen reacting with antibody can be calculated. Knowing the *in vitro* antigen-antibody combining ratios, one can estimate the amount of antibody involved in the accelerated elimination of antigen. From an analysis of the antigen elimination curves and the tissues of numerous animals, it has been possible to estimate the minimum rate of antibody synthesis necessary to evoke allergic lesions in the presence of excess antigen.

MATERIALS AND METHODS

Three solutions were used for intravenous injection:

(a) crystallized bovine plasma albumin (Armour and Co.) dissolved in sterile saline to form a 10 per cent solution with sufficient sodium bicarbonate to adjust the pH to 7.4.

(b & c) bovine plasma gamma globulin (Fraction II, Armour and Co.) dissolved in sterile saline to form 10 and 20 per cent solutions. The 20 per cent solution was employed for doses of bovine gamma globulin greater than 1 gram.

A total of 145 male albino rabbits weighing approximately 2 kilograms were employed in this study. Twelve of this number were bled and maintained under the same conditions as the experimental animals and sacrificed at the completion of the experiment. Their tissues were studied for lesions which might arise spontaneously. Each of the remaining 133 animals received a single intravenous injection of antigen as follows:

No. Rabbits	Dose, Gms.	Volume, ml.
	Bovine albumin (BSA)	
18	2	20
12	1	10
22	0.5	5.0
12	0.25	2.5
9	0.1	1.0
	Bovine gamma globulin (BGG)	
12	4	20
12	2	10
12	1	10
12	0.5	5.0
12	0.1	1.0
	None	
12		

All animals receiving bovine gamma globulin and the 12 controls were studied at Boston. Eleven of 18 rabbits receiving 2 gms. of bovine albumin and 12 of 22 receiving 0.5 gm. of bovine albumin were studied at Boston while the remaining animals receiving bovine albumin were observed at Baltimore.

In addition to these experimental animals, 23 other animals receiving 0.5 gm. of bovine albumin (13 at Boston and 10 at Baltimore) have been included to further demonstrate the relationship between the immune response and the development of allergic lesions.

Four ml. blood samples were removed from the marginal ear vein of all animals receiving bovine albumin, beginning on the second day after the antigen injection and continuing at 2 day intervals until the animals were sacrificed. Animals receiving bovine gamma globulin were bled on days 1, 2, 4, 6 and 8 and daily thereafter until the test for circulating bovine globulin was negative. Before sacrifice, another blood sample was withdrawn. Serum was removed from the specimens of clotted blood within 24 hours after collection and stored at −15°C until used for the quantitative serum antigen determinations to be described later.

Ordinarily, in studies on serum sickness, all animals are killed at the same time after antigen injection; that is, usually at 2 weeks. Previous experience has shown that individual animals vary considerably in their immune response and therefore their ability to eliminate antigen. Since the rapidity of antigen clearance is a measure of the immune response and moreover, since the allergic lesions are maximal at the time the serum antigen level has just become negative (4), it became clear that any study on the correlation of the degree of immune response of an individual animal with its ability to produce lesions necessitated sacrifice of that animal when the antigen level had just become negative.

In order to determine the time at which the antigen had completely disappeared from the circulation, animals were tested daily, after the 5th day, for the presence of circulating antigen. A rapid capillary precipitin method was devised for this purpose. Into a glass capillary tube (1.5 mm. x 100 mm., commonly used for melting point determinations) heparin solution ("Liquaemin," Organon, Inc.) was drawn for a distance of 2–3 mm. A drop of blood was obtained by pricking the rabbit's marginal ear vein, mixed directly with the heparin and drawn to three-fourths of the length of the tube. The capillary tubes containing blood samples from different rabbits were placed in modeling clay with gentle pressure to seal the bottom and then labeled with thin strips of adhesive tape. The tubes were centrifuged at 2,000 r.p.m. for 10 minutes in a wide test tube, the bottom of which was packed with clay. After centrifugation, each tube was broken at the red blood cell-plasma interphase; the plasma mixed with an equal volume of potent specific antiserum (a small drop on glazed paper) and this mixture drawn into the capillary tube which was then placed in a strip of modeling clay. After 30 minutes, at room temperature, as little as 5 micrograms of antigen nitrogen per ml. of test plasma gave a visible precipitate.

All animals were sacrificed when the capillary tube test for antigen became negative. As previously stated, in addition to indicating the appropriate time for sacrifice of the animals for analysis of lesions, the time at which the antigen is cleared in relation to dosage and time of injection provides valuable information on the magnitude of the immune response, for the rate of antigen clearance (catabolism) in the absence of antibody is remarkably similar for all animals (see references 4, 7 and present data). Therefore, differences in the duration of antigen in different animals can be used as an indicator of their relative capacity to produce antibody while antigen was present. For such comparative purposes, it has been found that the determination of the time of antigen disappearance can supplant the more difficult and time-consuming quantitative analysis of antigen clearance as a measure of immune response.

Quantitative immunochemical determinations for serum antigen by the use of calibrated pooled specific antiserum were performed usually on 3 animals for each of the following dosages of bovine albumin: 2 gm., 1 gm., and 0.25 gm.; and of bovine gamma globulin: 4, 2, 1, and 0.5 gm. The sera selected for analysis were from animals showing slow, medium, and fast rates of antigen clearance respectively. In addition, quantitative antigen elimination curves were obtained on 10 rabbits receiving 0.5 gm. bovine albumin.

On the day of sacrifice, i.e., when the antigen had just disappeared, the animals were killed by air embolism, weighed and immediately autopsied for histologic study. The tissues regularly examined included the lungs, heart, liver, pancreas, spleen, kidneys, stomach, intestine, and mesenteric lymph nodes. The spleen and kidneys were weighed. Tissues were fixed in 10 per cent formalin and in Zenker-formol solution and stained with hematoxylin-eosin.

EXPERIMENTAL RESULTS

A. The Effect of Various Doses of Bovine Albumin

1. Pathologic Findings

The lesions produced by bovine albumin have been described in detail elsewhere (4). They consisted of marked necrotizing arteritis found predominantly in the heart and lungs and to a lesser degree in the mesentery, spleen, liver, stomach, and hilum of the kidney; subendothelial cellular infiltrations in the aorta and pulmonary arteries; endocarditis; proliferative glomerulonephritis and granulomatous lesions in the lymphoid tissues of the spleen and lymph nodes. In addition to the diffuse proliferative glomerulonephritis ordinarily produced by albumin, there occurred, in 16 of 46 (35 per cent) animals with kidneys showing proliferative glomerular changes, prominent focal necrosis of the glomeruli (Figs. 1–4). In its early stage, this lesion consisted of pyknosis of the nuclei of cells of several adjacent capillar loops. In its mature form, large portions or even the whole glomerular tuft were completely destroyed and the glomerulus appeared as a structureless apparatus filled with blood. Compared to the proliferative alterations (consisting of hypertrophy and increase in the number of glomerular nuclei) these changes were never very widely disseminated. One of the interesting features of this lesion was its tendency to localization in the glomeruli nearest the cortico-medullary junction (Fig. 4), as indicated by the following data:

Rabbits treated with 0.5 gm. bovine albumin	Number of glomeruli with focal necrosis*	
	Inner half of cortex	Outer half of cortex
#551	2	0
#547	2	0
#545	2	0
#550	6	0
#546	6	0
#543	12	1
#553	56	13

* 600–700 glomeruli observed in each animal.

The data showing the incidence of glomerulonephritis and arteritis following different doses of bovine albumin are summarized in Table I and in Text-Figure 1. Marked vascular and renal lesions were observed following single injections of albumin at doses of 2, 1, and 0.5 gms., while a single injection of 0.25

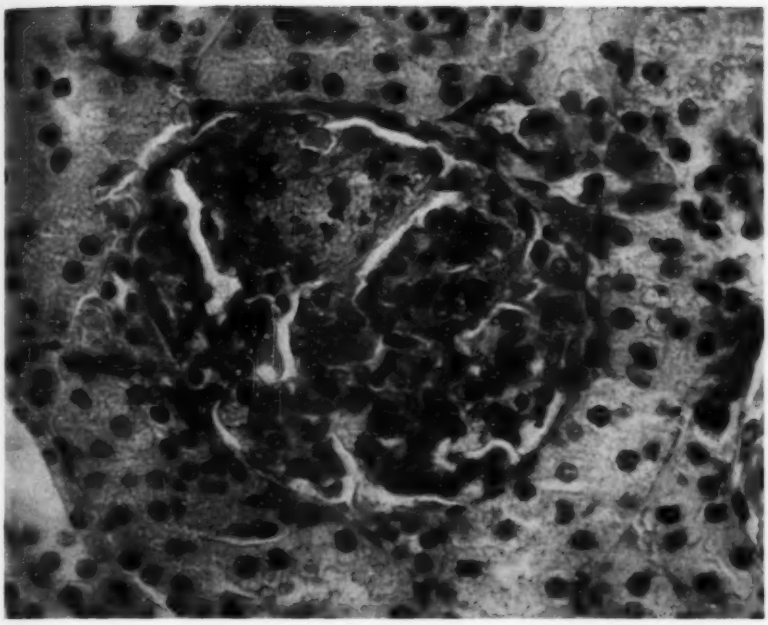


FIG. 1. Rabbit 553, receiving 0.5 gm. bovine albumin. Kidney. Glomerulus showing marked proliferation and early necrosis of glomerular loops (upper pole). Hematoxylin and eosin. × 500.

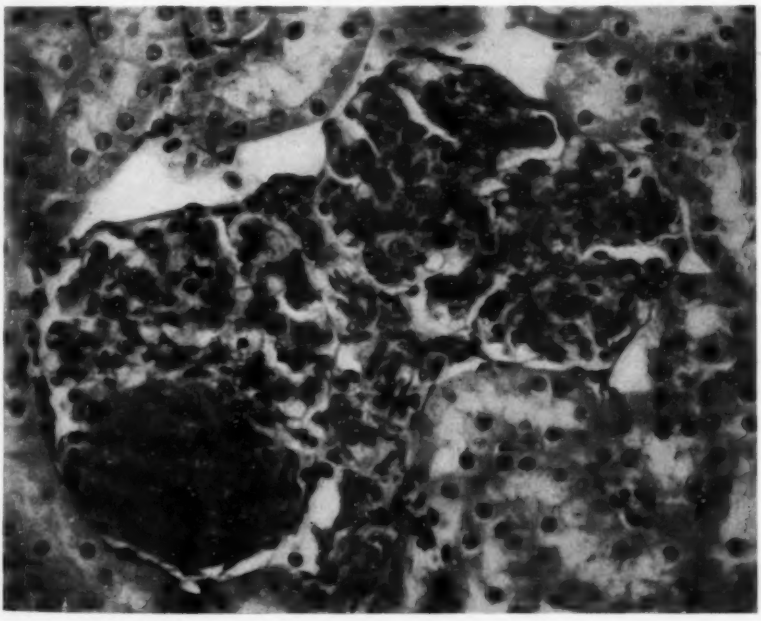


FIG. 2. Rabbit 545, injected with 0.5 gm. bovine albumin. Kidney. Two glomeruli showing marked proliferative changes. There is focal necrosis of the glomerulus on the left. Hematoxylin and eosin. × 350.

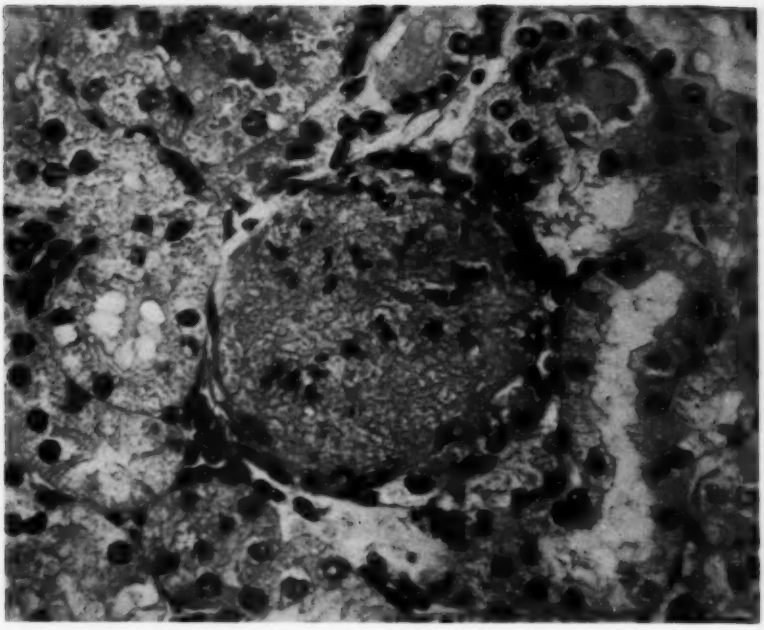


FIG. 3. Rabbit, 553, injected with 0.5 gm. bovine albumin. Kidney. Complete glomerular necrosis. Hematoxylin and eosin. × 500.

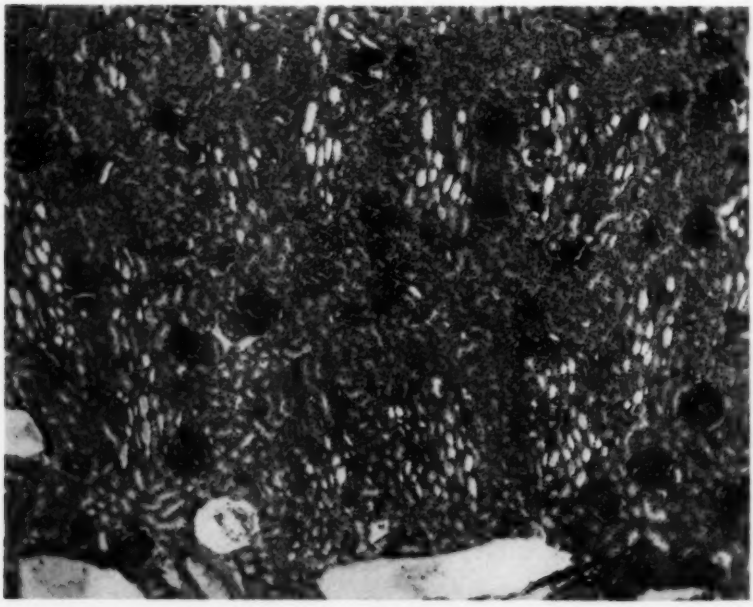


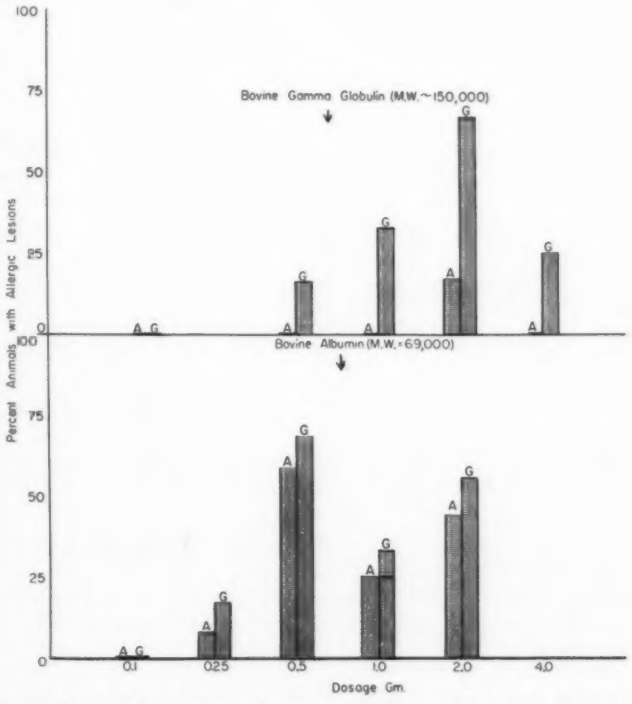
FIG. 4. Rabbit 553, injected with 0.5 gm. bovine albumin. Kidney. Low power of cortex showing proliferative and focal necrotizing glomerulitis; the latter is concentrated in the lower half of the photograph which represents the cortex nearest the medulla. Hematoxylin and eosin. × 50.

TABLE I

Relationship Between Dosage of Antigen and Development of Allergic Lesions

Dosage, Gms.	No. of Animals	Animals with any Lesion[1]		Animals with Arteritis		Animals with Glomerulonephritis	
		No.	%	No.	%	No.	%
Bovine Albumin							
2	18	10	56	8	44	10	56
1	12	4	33	3	25	4	33
0.5	45	33	73	26	58	30	67
0.25	12	4	33	1	8	2	17
0.1	9	0	0	0	0	0	0
Bovine Gamma Globulin							
4	12	3	25	0	0	3	25
2	12	8	67	2	17	8	67
1	12	4	33	0	0	4	33
0.5	12	2	17	0	0	2	17
0.1	12	0	0	0	0	0	0

[1] Additional experience with bovine albumin at Boston. Glomerulonephritis and splenic lesions graded + to 3+; nec. = necrotizing.



TEXT-FIG. 1. Relationship between dosage of antigen and development of allergic lesions (arteritis (A) and glomerulonephritis (G)). After data of Table I.

TABLE II

Serum Antigen Levels After Intravenous Injection of Varying Dosages of Bovine Albumin

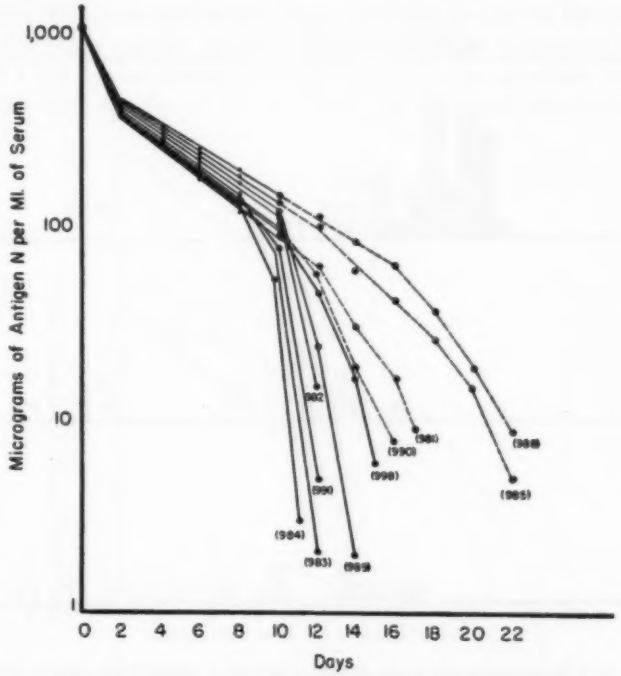
Rabbit No.	Dosage, BSA Gms.	Mg. Antigen Nitrogen per ml. of Serum on Days After Injection																
		2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
974	2	1.48	1.02	.730	.570	.428	.131	.002											
972		1.52	1.14	.780	.570	.376	.256	.156	.090	.054	.025	.008	tr.						
971		1.46	1.04	.790	.620	.468	.344	.232	.156	.094	.060	.038	.026	.010	.008	.006			
439	1	.720	.480	.376	.280	.196	.088	tr.											
446		.720	.460	.364	—	.170	—	—	.046	.026	.010	.004*							
435		.610	.480	.368	—	.265	.214	.150	.119	.094	.071	.058	.042	.030	.021	.014	.009	.006	.002*
984	0.5	.384	.256	.188	.136	.050	.003*												
982		.430	.276	.240	.172	.110	.015												
983		.410	—	.204	.154	.075	.002												
991		.384	.300	.227	.154	.094	.005												
989		.408	.290	.200	.156	.090	.026	.002											
978		.392	.300	.232	.134	.100	.048	.017	.006*										
990		.360	.273	.196	.162	.110	.059	.018	.008										
981		.396	.238	.173	.126	.080	.068	.031	.017	.009*									
985		.372	.260	.234	.178	.128	.096	.057	.042	.026	.015	.005							
986		.384	.300	.230	.180	.138	.104	.074	.058	.036	.019	.009							
452	0.25	.172	.134	.094	.038	.007													
399		.220	.142	.098	.061	.046	.022	.005*											
371		—	.164	.106	.085	—	.043	.030	.021	.014	.005*								

* Antigen level one day earlier than indicated.

gm. of bovine albumin produced a lower incidence of lesions, consisting chiefly of moderate proliferative glomerulonephritis and granulomatous alterations of the spleen. No lesions were observed following an injection of 0.1 gm. of bovine albumin.

2. Histologic and Immunologic Correlations

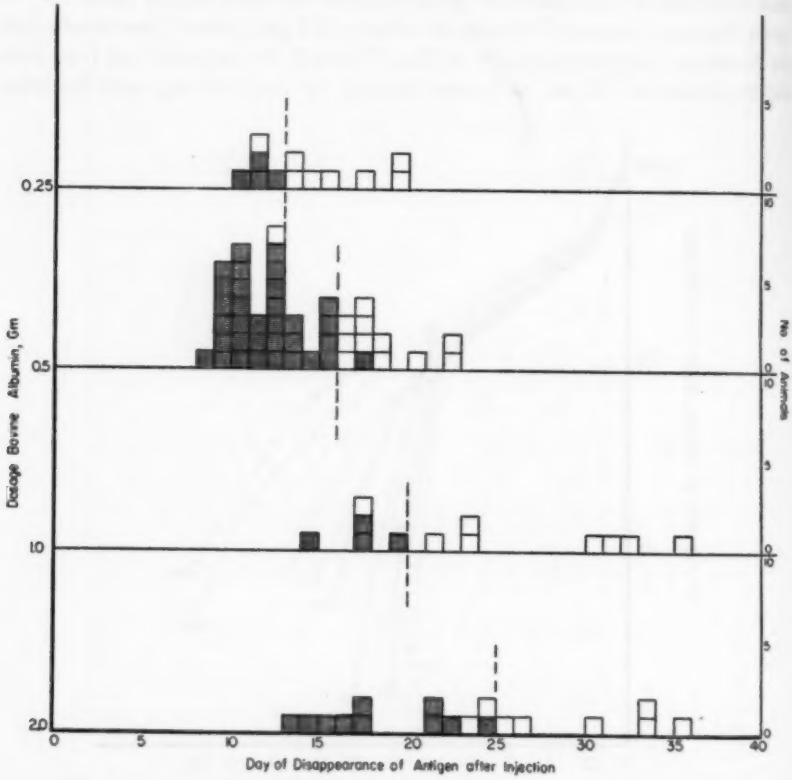
The results of quantitative serum antigen determinations on selected animals receiving different doses of bovine albumin are tabulated in Table II. The blood clearance curves of 10 animals receiving 0.5 gm. doses of bovine albumin are presented diagrammatically in Text-Figure 2. As indicated, all 6 animals which eliminated 0.5 gm. of bovine albumin by the 15th day after injection



TEXT-FIG. 2. Blood clearance and lesions with 0.5 gm. bovine albumin. After data of Table II. Each line represents the serum antigen clearance of a single animal. Antigen clearance between injection and day 2 is due to equilibration; after day 2 until time of more rapid elimination, the clearance is the result of catabolism. Note the similarity of the equilibration and catabolic phases of antigen elimination of the individual animals. The third phase of more rapid antigen elimination is due to antibody response. Note the variation in antibody response. Animals developing allergic lesions are represented by solid lines; those without lesions appear as broken lines.

developed lesions. However, no lesions attributable to allergy were present in the 4 animals which eliminated the antigen at a later time.

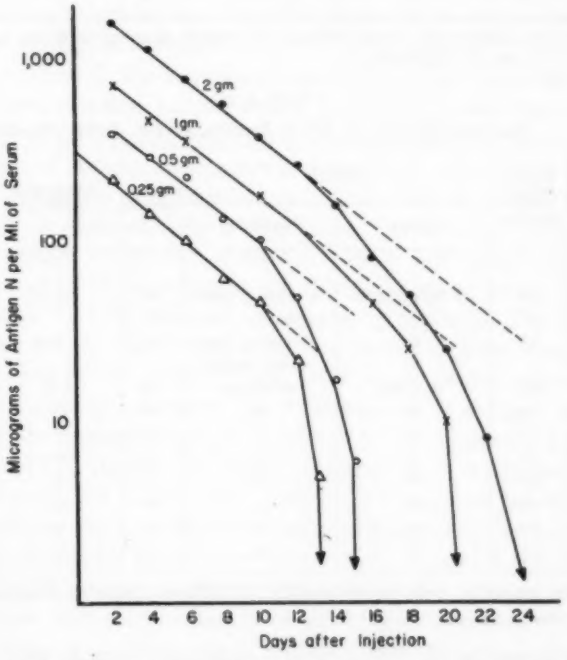
The data showing the relationship between antigen clearance and the occurrence of lesions for all doses of bovine albumin are given in Text-Figure 3. As shown there, for each twofold increase in dosage of bovine albumin, the limiting time for the elimination of this antigen by antibody formation at a rate consistent with the production of lesions was increased by approximately 4–5



TEXT-FIG. 3. Relationship between dosage of albumin, rapidity of antigen clearance and development of allergic lesions. Each animal is represented by a block. Shaded blocks represent animals developing allergic lesions. Vacant blocks are animals not exhibiting allergic lesions. Note the relationship at each dosage between rapidity of antigen clearance (immune response) of a given animal and development of lesions. Note also that an increase in dosage causes a regular increase in the maximum time of antigen clearance consistent with the development of lesions. The broken vertical lines represent the slowest rate of antigen clearance (minimum immune response) consistent with the development of lesions in the series of animals tested.

days. For 0.5 gm., the length of time was 15 days; for 1 gm., 20 days, and for 2 gm., 25 days. This result is not surprising in view of the fact that the half-life (rate of catabolism) of bovine albumin at all dosages is approximately 4 to 5 days (Text-Fig. 4). If a critical amount of this antigen is necessary to interact with antibody for lesions to occur, this amount will be present 5 days later when the dosage of albumin is doubled.

In Table III are recorded the relationships between the rapidity of antigen disappearance (immune response) and the occurrence of lesions in animals comprising a separate experiment carried out in Boston. Of 13 animals receiving 0.5 gm. bovine albumin, 12 cleared the antigen before the 15th experimental day; 11 of the 12 showed lesions. One animal clearing the antigen on the 16th day did not develop lesions. In Table IV is recorded a similar study carried out in Baltimore with rabbits from a different source. Of the 10 animals receiving 0.5 gm. of bovine albumin, only 3 cleared the antigen by the 15th day; these 3 had marked lesions. Of the remaining 7 animals which cleared the antigen after the 17th day, 6 had no lesions and the other had only a granu-



TEXT-FIG. 4. Median blood clearance of graded doses of bovine albumin. After data of Table II. Note the family of curves. Broken lines indicate projected rate of antigen clearance by catabolism alone.

TABLE III

Histologic Alterations in Rabbits Receiving 0.5 Gm. Bovine Albumin[1]

Rabbit No.	Day of Antigen Disappearance	Arteritis		Endocarditis	Glomerulonephritis	Granulomatous Lesions	
		Intimal	Necrotizing			Spleen	Lymph Nodes
Exp. 9H							
321	8	+ aorta, lung	+ heart	0	+++ nec.	+++	+
322	9	+ aorta	0	0	+++ nec.	++	0
325	9	+ aorta, lung	0	0	+	+++	0
326	9	0	+ heart	0	+++ nec.	+++	0
329	9	+ aorta, lung	0	+	++ nec.	+++	+
330	9	+ aorta	0	0	+++ nec.	+++	+
323	10	+ lung, aorta	+ heart	0	+++ nec.	+++	0
324	10	+ lung, aorta	0	0	+	+++	+
327	10	+ lung	0	+	+	+++	+
328	12	0	0	0	++	+++	0
Exp. 11H							
511	9	+ aorta, lung	+ liver, lung	+	+++	+++	–
509	12	0	0	0	0	0	0
512	16	0	0	0	0	0	0

[1] Additional experience with bovine albumin at Boston; glomerulonephritis and splenic lesions graded + to 3+; nec. = necrotizing.

TABLE IV

Histologic Alterations in Rabbits Receiving 0.5 Gm. Bovine Albumin[1]

Rabbit No.	Day of Antigen Disappearance	Arteritis		Endocarditis	Glomerulonephritis	Granulomatous Lesions	
		Intimal	Necrotizing			Spleen	Lymph Nodes
Exp. 9J							
11	13	+ lung, aorta	+ heart, pancreas	+	+	0	0
2	15	0	+ heart, pancreas, spleen	+	+++	0	0
3	15	+ lung	+ spleen	0	++	0	0
14	16	0	0	0	0	0	0
10	17	0	0	0	0	+++	0
12	17	0	0	0	0	0	0
13	17	0	0	0	0	0	0
4	18	0	0	0	0	0	0
5	18	0	0	0	0	0	0
1	21	0	0	0	0	0	0

[1] Additional experience with bovine albumin at Baltimore; experiment undertaken with Dr. James A. Shaka.

lomatous reaction in the spleen. These experiences indicate that differences in the ability to develop an adequate immune response in the presence of circulating antigen are one of the most important factors contributing to the marked variation in the development of lesions.

TABLE V

The Relationship Between the Rate of Immune Response and the Development of Allergic Lesions

Rabbit No.	Amount of Antigen Eliminated During Immune Phase, mg AtN/ml Serum	Total Body Antigen Eliminated During Immune Phase; mg AtN	Total AbN, mg	Length of Ab-At Reaction, Days	Average AbN Synthesized per Day, mg	Presence of Allergic Lesions
BSA 0.5 gm						
984	.082	13.1	65.5	3 (8–11)	22	+
982	.070	11.2	56.0	2 (10–12)	28	+
983	.082	13.1	65.5	4 (8–12)	16	+
991	.086	13.8	69.0	4 (8–12)	17	+
989	.054	8.6	43.0	4 (10–14)	11	+
978	.052	8.3	41.5	5 (10–15)	8	+
990	.033	5.3	26.5	4 (12–16)	7	0
981	.023	3.7	18.5	5 (12–17)	4	0
985	.010	1.6	8.0	4 (18–22)	2	0
986	.021	3.4	17.0	6 (16–22)	3	0

By calculating the amount of antigen eliminated by antibody during the immune phase of antigen clearance, it is possible to estimate the amount of antibody involved in the interaction with antigen for the production of serum sickness. The amount of antigen involved in the immune phase was calculated by stepwise analysis of the immune phase of the antigen elimination curves, i.e., the decrease of serum antigen over a 2 day period was substracted from the amount of antigen which would have been present if the semi-logarithmic catabolic phase of antigen elimination had continued. From each determined point of antigen serum concentration, a line was drawn parallel to the pre-immune catabolic phase of antigen elimination. Differences between this projected serum antigen concentration and the actually determined serum antigen value were accepted as representing the change in serum antigen concentration attributable to immobilization of antigen by antibody. The decreases in serum antigen concentration attributable to antibody were summated and multiplied by 160 which represents the total serum protein space of a 2 kg. rabbit.[5] This figure approximates the total amount of antigen in the body eliminated by the immune response. Assuming a combining ratio by weight of Ab and At to be 5:1, the amount of antibody involved in the immune response can be estimated. The results of an analysis of 10 animals receiving 0.5 gm. bovine albumin on which quantitative antigen clearance curves were obtained are summarized in Table V. As shown there, of the 6 animals showing lesions, all had eliminated antigen in the amount greater than approximately 8 mg. antigen nitrogen. This is equivalent to the production of approximately 40 mg. antibody nitrogen or a critical rate of antibody formation (and interaction) of

[5] This factor is obtained following equilibration of the antigen by dividing the total amount of antigen injected less that which has been catabolized, by the serum antigen concentration.

approximately 8 mg. antibody nitrogen per day for a 5 day period (see rab. # 978, Table V).

B. The Effect of Various Doses of Bovine Gamma Globulin

1. Pathologic Findings

The lesions produced by a single injection of gamma globulin were almost exclusively confined to the glomerulus of the kidney. This lesion consisted of proliferative glomerulonephritis; only one animal showed focal glomerular necrosis. In general, the glomerular lesions were much less striking than those produced by bovine albumin. Four of the 17 animals developing glomerulonephritis following various doses of globulin showed slight granulomatous splenic lesions, and 2 showed subendothelial infiltrations in the pulmonary arteries. No necrotizing arteritis was observed in any of the animals treated with bovine gamma globulin.

The highest incidence of glomerulonephritis (67 per cent) was observed in animals receiving 2 gm. of bovine gamma globulin (Table I and Text-Fig. 1). Animals receiving 4 gm. of bovine gamma globulin showed a lower incidence of glomerular lesions and the lesions were not as severe as with the 2 gm. dose. Below 2 gms., the incidence of lesions diminished proportionately with the dosage so that following 0.1 gm. no lesions were observed.

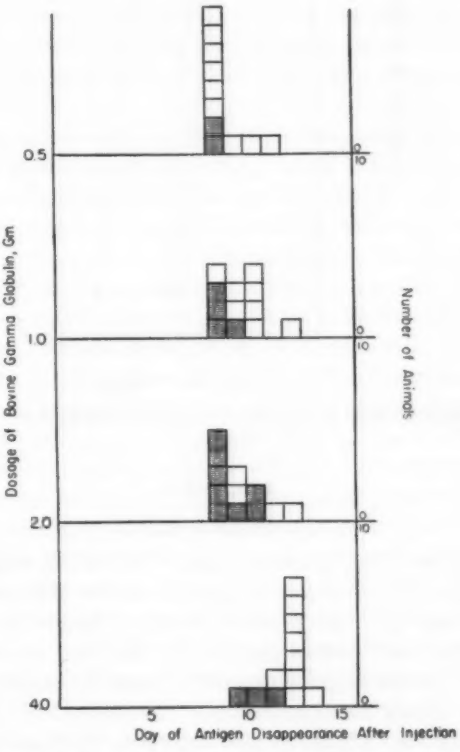
2. Histologic and Immunologic Correlations

The relationships between the rate of antigen clearance and the development of lesions following 4, 2, 1, and 0.5 gm. doses of bovine gamma globulin are recorded in Text-Figure 5. Only the animals showing a rapid clearance of antigen indicative of a prompt immune response developed lesions. The time of antigen clearance consistent with an immune response capable of producing lesions was progressively longer with increasing doses of bovine gamma globulin. However, in view of the fact that the catabolic half-life of bovine gamma globulin is 2 days (Text-Fig. 6), instead of 4–5 days as is the case with albumin, the differences between the time of clearance of varying doses of bovine gamma globulin were not as great as with graded doses of bovine albumin. The longest time of clearance of 4 gm. of bovine gamma globulin consistent with an immune response adequate to produce lesions was 11 days after injection; for 2 gms., 10 days; for 1 gm., 9 days, and for 0.5 gm., 8 days.

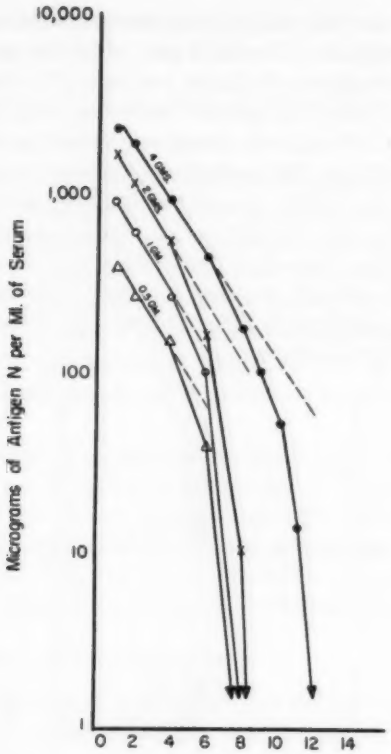
The median blood clearance for each dose of bovine gamma globulin is presented diagrammatically in Text-Figure 6. Text-Figure 6 indicates that the median blood clearance of 4 gms. of bovine gamma globulin was much slower than the clearance of smaller doses of this antigen, apparently due to the fact that the "immune" phase of elimination of 4 gms. of bovine gamma globulin

began 2 days later than with smaller doses and persisted at least 1 day longer. The poor antibody response following 4 gms. of bovine gamma globulin tends to explain the lower incidence of allergic lesions at this dosage.

From calculations made in a manner similar to those described for bovine albumin, the amount of antibody nitrogen involved in the interaction with bovine gamma globulin for the production of lesions was estimated to be approximately 24 mg., equivalent to a rate of antibody synthesis of 6 mg. antibody nitrogen per day over a 4 day period. Since quantitative antigen determinations were performed on only a few animals receiving bovine gamma globulin, the amount of antibody required for the production of lesions by this antigen may not be significantly different from that found for the occurrence of lesions following bovine albumin.



TEXT-FIG. 5. Relationships between dosage of bovine gamma globulin, duration of serum antigen and development of allergic lesions. Those animals with allergic lesions are represented by shaded blocks; those without lesions by unshaded blocks.



TEXT-FIG. 6. Median serum antigen levels following varying amounts of bovine gamma globulin.

DISCUSSION

Pathologic Findings

Differences in the distribution of allergic lesions following bovine albumin on the one hand and bovine gamma globulin on the other were first noted by Hawn and Janeway (3). These workers observed the paucity of arterial lesions in animals treated with bovine gamma globulin and concluded that bovine gamma globulin produced predominantly glomerular lesions while bovine albumin gave rise chiefly to marked arterial alterations. The present study and other studies from this laboratory and elsewhere (9, 5) are in agreement with the finding that bovine gamma globulin produces lesions more or less confined to the glomeruli of the kidneys. However, the present work and earlier studies by us (4, 8) have shown that bovine albumin often produces marked glomerular

lesions as well as prominent injury to the arterial system. In our experience the glomerular lesions produced by bovine albumin have been considerably more marked than those observed following bovine gamma globulin (references 4, 8, 10, and present data). The failure of Hawn and Janeway to have observed glomerular lesions in animals treated with albumin might have been due to the fact that their animals were sacrificed at weekly intervals rather than individually, at a time when the antigen has just disappeared from the circulation and the lesions are maximal.

The most notable difference between the effects of single intravenous injections of bovine gamma globulin and bovine albumin is the paucity of arterial lesions following injections of the former antigen. It is possible that the more rapid catabolism and the shorter duration of bovine gamma globulin in the body might preclude the development of marked arterial lesions (9). It is of interest that arterial lesions comparable to those produced by albumin may be elicited by bovine gamma globulin when adequate levels of this antigen are maintained over a longer period of time than those occurring following a single injection (9, 10).

While the glomerular lesions ordinarily observed following an injection of foreign protein are of the proliferative type, other investigators have shown that focal necrosis of the glomerular tuft may also occur (11, 12). In the present study, focal necrotizing glomerular capillary lesions were most frequent in animals treated with bovine albumin where damage to the larger vessels is a common finding. Several considerations suggest that the development of glomerular necrosis is related in large part to the severity of the allergic reaction. Firstly, this lesion was only observed in animals with the most marked proliferative changes in the glomeruli. Secondly, the occurrence of this lesion was most frequent in animals showing a marked immune response, as measured by the rapidity of antigen elimination. Of 17 animals eliminating a 0.5 gm. dose of bovine albumin between the 8th and 11th days and developing proliferative glomerular alterations, focal necrotizing glomerular lesions were observed in 11. Of 13 animals clearing the antigen between the 12th and 15th days and developing proliferative glomerular lesions, focal necrosis of the glomerular tufts was observed in 2.

The focal glomerular lesions tended to be situated predominantly in the glomeruli bordering the cortico-medullary junction. The reason for this distribution is not clear. Rich (13) has noted a similar distribution of the proliferative glomerular lesions of the late stages of human cases of "lipoid" nephrosis. It is possible that the difference in susceptibility to injury of the glomeruli located at various levels in the cortex might be related to differences in hemodynamics.

It is clear that in experimental hypersensitivity, at least two types of lesions may appear; namely necrotizing arteritis, and a glomerular lesion which may

be present as a necrotizing glomerulitis and as a proliferative type of glomerular change. Although human periarteritis nodosa takes its name from the spectacular vascular changes, both proliferative and necrotizing glomerular alterations frequently form an integral part of this disease process (14, 15). Clearly the glomerular lesions produced by experimental hypersensitivity under conditions which often promote the development of arterial lesions must be closely related if not identical pathogenetically to the glomerular alterations of human periarteritis nodosa. The glomerular lesions of experimental hypersensitivity have often been compared to the glomerulonephritis in humans following streptococcal infection (12, 16–19). Although post-scarlatinal glomerulonephritis may be founded in allergy, the possibility exists that glomerulonephritis as an anatomical entity might result from a variety of injuries.

Immunologic and Histologic Sequences

The results of the present study indicate that the minimum dose of bovine albumin effective in producing allergic lesions when given as a single intravenous injection into rabbits weighing 2 kg. is 0.25 gm.; the minimum dose of bovine gamma globulin is 0.5 gm. The latter finding is in agreement with the work of Moll and Hawn showing that the minimum dose of bovine gamma globulin effective in producing lesions was 0.25 gm. per kg. of body weight. Since the molecular weights of bovine albumin and gamma globulin differ by approximately twofold (69,000 and 150,000 respectively), the twofold difference in the minimum doses of bovine albumin and globulin effective in producing lesions may be a function of antigen-antibody molecular equivalents.

Previous work has indicated a temporal correlation between the rate of antigen disappearance and the development of lesions (4, 8). The rate of antigen clearance from the blood is determined by the rate of antigen catabolism and the rapidity of formation of antibody effective in antigen immobilization (7). Since the rate of normal catabolism of a given antigen varies only slightly in individual animals, it is apparent that differences in the blood clearance of antigen are determined largely by the degree of immune response. Since a certain level of antigen-antibody is necessary for the production of allergic lesions, the presence or absence of allergic lesions can be correlated with the time that it takes for the antigen to be cleared from the blood. For instance, following 0.5 gm. of bovine albumin, most of the animals which cleared the antigen by the 15th day produced lesions; those in which circulating antigen was present after the 15th day seldom exhibited lesions.

When the dosage of antigen is increased twofold, the persistence of antigen in the body is increased by a factor which is dependent on the half-life at which the antigen is normally catabolized. With bovine albumin, which has a catabolic half-life of 5 days, each twofold increase in dosage results in the same

circulating level of antigen occurring 5 days later than with the preceding dose. Consequently, the maximum length of time bovine albumin may persist in the body (with an immune response adequate to produce lesions) is lengthened by approximately 5 days with each twofold increase in dosage. Since the half-life of bovine gamma globulin is approximately 1 to 2 days, the differences in the duration of twofold increases in antigen dosage are not as great as with albumin.

It is of interest that a 4 gm. dose of bovine gamma globulin produced on the average a smaller antibody response than did a 2 gm. dose. This small immune response was reflected in a lower incidence of lesions at the 4 gm. dose. Others have reported the occurrence of "immune unresponsiveness" after a single injection of polysaccharide antigen in the mouse (20) and after repeated large injections of protein antigens in the rabbit (21). The mechanism by which "immune unresponsiveness" is produced is not entirely clear, although in the case of polysaccharide antigens which are poorly catabolized, persistence of the antigen seems to play a major role (22).

From a stepwise analysis of the antigen elimination curves, information has been obtained concerning the amount of antibody required for the production of lesions. This amount has been estimated as 40 mg. Ab N for the bovine albumin treated animals and 24 mg. Ab N for animals treated with bovine gamma globulin. In view of the small numbers of animals in which these determinations were made and the indirect method by which the values were obtained, these figures can only be considered as an approximation. It is nevertheless apparent that the amount of antibody required for the production of serum sickness is vastly greater than that required for anaphylactic shock and the Arthus reaction (23–26). It is possible that the amount of antibody required to produce allergic lesions might vary with the quality of antibody produced. Further, since these values represent the entire response of the animal it is still not clear what amount of antibody is actually involved in the production of a single lesion in the cardiovascular system.

The difficulties involved in the production of experimental allergic lesions with regularity are well known. This has been attributed to "individual variation." The present data demonstrate that one of the most important variables is the ability of the animal to produce sufficient antibody while antigen is still present. This is clearly shown by the marked variations in the immune responses and consequently the occurrence of allergic lesions in animals obtained from different sources.

From a practical point of view, the capillary tube method for the determination of circulating antigen is extremely valuable. This method permits quick determination of the time of antigen clearance. This finding relates information as to the immune response of the animal, allows for correlation of the immune response and occurrence of lesions and in experiments where it is only desired

to study the histologic lesions, animals with slow antigen clearance (i.e., poor immune response where lesions are unlikely to develop) can be dispensed with and only the animals likely to have lesions studied in histologic detail.

SUMMARY

The present study was undertaken to determine the relationships between the chemical nature of the antigen, antigen dosage, immune response and the development of allergic lesions. Rabbits weighing approximately 2 kg. were injected with graded doses of crystalline bovine gamma globulin, given as a single intravenous injection.

The minimum dosage of bovine albumin conducive to the development of allergic lesions was 0.25 gm. in a 2 kg. rabbit; for bovine gamma globulin, this dosage was 0.5 gm. The critical amount of antibody involved in the production of lesions has been *estimated* as 40 and 24 mg. Ab nitrogen respectively for the above two antigens.

The data indicate that differences in the degree of immune response in the presence of circulating antigen are one of the most important factors contributing to the marked variation in the development of allergic lesions.

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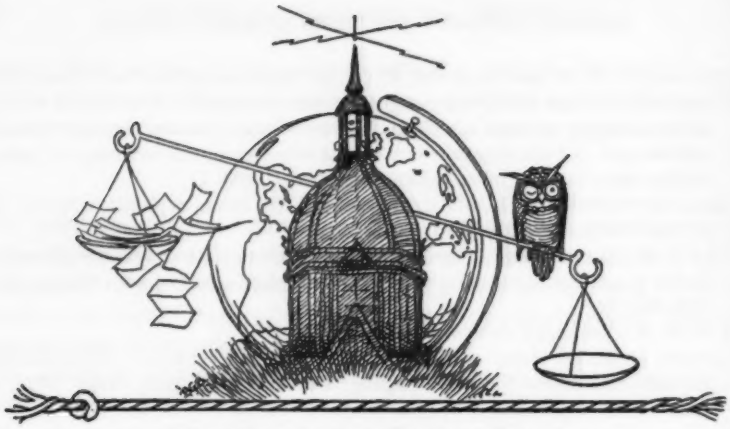

BOOK REVIEWS

History of Nursing Source Book. By ANNE L. AUSTIN. 480 pp., $7.50. G. P. Putnam's Sons, New York, N. Y.

This book, which belongs to the Modern Nursing Series, is intended as a companion volume to textbooks on the history of nursing. The author, formerly professor of nursing at Western Reserve University, presents literary source material from early civilization to the founding of the first school of nursing in America in 1873. The individual chapters are introduced by a short outline characterizing the period and the sources used; the readings are preceded by short notes, and each chapter ends with a concluding statement. The scope of the work is wide. A description of hot applications from the Hippocratic works finds its place as does Dickens' portrayal of Mrs. Gamp and Mrs. Prig. The whole second part is devoted to the life of Florence Nightingale and the new profession of nursing she established, especially through the school at St. Thomas's Hospital, London. The last part deals with nursing in the western hemisphere. The "Notes" give the provenience of the readings, and the book concludes with a rather extensive bibliography and a good index. It promises to serve its educational purpose well and to prove of interest also to many readers outside the nursing profession.

OWSEI TEMKIN

Essentials of Clinical Proctology, 3rd edition. By MANUEL G. SPIESMAN AND LOUIS MALOW. 316 pp., $8.75. Grune & Stratton, New York, N. Y.

This relatively brief consideration of rectal surgery would appear to be directed principally toward the office practice of this specialty. The table of contents is encyclopaedic but the discussion is frequently less thorough than might seem desirable. This is particularly noted, for example, in the consideration of malignancies, rectal prolapse, ulcerative colitis and anal stenosis. It should be stated that this objection does not apply to many of the chapters, particularly those dealing with the more common rectal disorders (fissure, fistula, abscess), these topics being well covered. A rather comprehensive discussion of the authors' experiences with the pecten band is included.

This book would seem to find its chief value as a reference text for the physician who is called upon to treat certain rectal conditions in his office practice or the surgical house officer in training. It is well suited to these purposes.

JAMES R. CANTRELL

Some Milestones in the History of Hematology. By CAMILLE DREYFUS. 87 pp., $4.50. Grune & Stratton, New York, N. Y.

Dr. Dreyfus' small volume does not constitute a formal history of hematology, but rather contains a personalized account of the evolution of our present knowledge of the blood. Initially many of the classical and Renaissance concepts of blood and its significance are set forth and those serve to dramatize the later contributions of systematic clinical observation coupled with improved microscopic and histologic techniques. Special mention is made of the early history of leukemia, polycythemia vera and chronic hemolytic anemia. A final chapter is devoted to the contributions of Georges Hayem.

Thus has Dr. Dreyfus provided us with refreshing and sobering backward glances with which to temper our current concepts of hematology.

ALBERT H. OWENS, JR.

Annual Review of Medicine, Vol. 8, 1957. Edited by DAVID A. RYTAND. 530 pp., $7.00. Annual Reviews, Inc., Stanford, California.

As in previous years, the new *Annual Review of Medicine* consists of a series of articles, varying in length and quality, on a large number of topics. The book is presumably intended both for browsing and for reference, and is aimed at physicians interested in maintaining at least a dilettante's contact with medicine in its broadest sense. Reviews like Buxton's on obstetrics or Ashton's on retrolental fibroplasia should prove interesting to any intelligent physician, although most "Annual Review" readers are probably not in those specialties concerned with direct practical application of such material. On the other hand, the volume contains some fine summarizations (such as that by Kass and Finland on the role of adrenocortical hormones in the management of infections) which should have widespread implications for many readers.

Some of the authors (happily few in number) have merely abstracted a lot of recent literature in unimaginative fashion, thus producing "reviews" useful primarily as sources of references. Other authors (Crosby on "The Red Cell and Some of Its Problems", for example) have also summarized a lot of recent literature, but in a manner which fuses progress reports and previously available data into a superb and comprehensive statement of the current status of a field.

There are a few new features. For the first time, a chapter is devoted entirely to the clinical application of radioactive isotopes. There are interesting and valuable special chapters on the prophylaxis of infectious diseases, pneumoconiosis, and the development of tolerance to, and physical dependence on, opiates, barbiturates, and alcohol. The usual chapter on neoplastic diseases is unfortunately missing, and lack of space has forced omission of the customary "Annotated List of Reviews" by the MacKays.

LOUIS LASAGNA

Hutchinson's Clinical Methods, 13th edition. By DONALD HUNTER AND R. R. BOMFORD. 452 pp., $6.00. J. B. Lippincott Co., Philadelphia, Pa.

Clinical Methods has enjoyed an exceptional publishing history since its first edition in 1897. The thirteenth edition contains the traditionally concise and authoritative descriptions of those established methods of clinical investigation which may be successfully applied at the bedside. The initial chapter is devoted to a general consideration of the history and physical examination. Subsequent chapters deal with a more detailed consideration of the examination of each physical system and with the procedures for the proper examination of blood, urine and other biologic fluids. An appendix contains data on weights and measures and the chemical constitution of various laboratory reagents.

The material is well organized, well written and amply illustrated. No discussion of differential diagnosis is attempted. The authors, however, stress the importance of a systematic and thorough investigation of each case as the necessary prerequisite to a correct diagnosis.

ALBERT H. OWENS, JR.

Treatment of Burns. By CURTIS P. ARTZ AND ERIC REISS. 250 pp., $7.50. W. B. Saunders Co., Philadelphia, Pa.

This recent addition to the already sizeable literature on burns is based upon experience gained in recent years by the Surgical Research Unit of the Brooke Army Medical Center.

The authors have advisedly included in their discussion not only thermal but all types of burns. The care of the burned patient is covered in adequate detail from the acute phase of local wound care and fluid and electrolyte disturbance to the late problems of grafting, metabolic disorders, and complications. Technical discussions of dressing, grafting, etc. are included. The consideration of these topics is not exhaustive but is adequate and this brevity enhances its practical value as a reference text. Selected references are provided. The text is well illustrated, the print large and easily readable and the paper is of good quality.

While little new material is presented, this book should prove useful as a reference for physicians called upon to carry out burn therapy.

JAMES R. CANTRELL

BOOKS RECEIVED FOR REVIEW

The Chemistry and Biology of Purines (Ciba Foundation Symposium). Edited by G. E. W. WOLSTENHOLME AND CECELIA M. O'CONNOR. 327 pp., $9.99. Little, Brown & Co., Boston, Mass.

Clinical Proctology, 2nd edition. By J. PEERMAN NESSELROD. 296 pp., $7.00. W. B. Saunders Co., Philadelphia, Pa.

Developmental Abnormalities of the Eye, 2nd edition. By IDA MANN. 419 pp., $15.00. J. B. Lippincott Co., Philadelphia, Pa.

Fluid and Electrolytes in Practice, 2nd edition. By HARRY STATLAND. 229 pp., $6.00 J. B. Lippincott Co., Philadelphia, Pa.

Goepp's Medical State Board Questions and Answers, 9th edition. By HARRISON F. FLIPPIN. 569 pp., $8.00. W. B. Saunders Co., Philadelphia, Pa.

Gout, 2nd edition. By JOHN H. TALBOTT. 205 pp., $6.75. Grune & Stratton, New York, N. Y.

William Harvey. His life and times; his discoveries; his methods. By LOUIS CHAUVOIS. 271 pp., $7.50. Philosophical Library, New York, N. Y.

The Infectious Diseases of Domestic Animals. With Special Reference to Etiology, Diagnosis and Biologic Therapy, 3rd edition. By WILLIAM ARTHUR HAGAN AND DORSEY WILLIAM BRUNER. 968 pp., $10.50. Cornell University Press, Ithaca, New York.

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Principles of Microbiology, 3rd edition. By CHARLES F. CARTER AND ALICE LORRAINE SMITH. 665 pp., $5.00. The C. V. Mosby Co., St. Louis, Mo.

Regulation and Mode of Action of Thyroid Hormones (Ciba Foundation Colloquia on Endocrinology Volume X). Edited by G. E. W. WOLSTENHOLME AND ELAINE C. P. MILLAR. 311 pp., $8.50. Little, Brown & Co., Boston, Mass.

The Specialties in General Practice, 2nd edition. Edited by RUSSELL L. CECIL AND HOWARD F. CONN. 780 pp., $16.00. W. B. Saunders Co., Philadelphia, Pa.